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PLANT PRODUCTION ON THE FLADEN GROUND

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(Text-figs. 1–19)

INTRODUCTION

The quantitative study of phytoplankton production may be pursued in many ways, but these ways can be divided into two general methods of approach. There is, first, the direct estimation of a production rate for a particular sample of the population; for example, the light-dark bottle technique for measuring oxygen production (Gaarder & Gran, 1927; Riley, 1939) and the new ^{14}C technique (Steeman Nielsen, 1952). These estimates are made under conditions which must be, to some extent, artificial. Secondly, there is the direct estimation of relevant variables in the sea (phosphate, oxygen, chlorophyll concentration, etc.) from which production is calculated on the basis of hypotheses about the behaviour of phytoplankton. These hypotheses are, of necessity, simplifications of a mass of laboratory experiments and of previous field work. Riley, Stommel & Bumpus (1949) give a full account of this approach and of the difficulties involved in it.

Since it is, in practice, impossible to use all methods at the same time, a choice of techniques must be made. The second approach was chosen in the investigations here described, for the following reasons: (*a*) the estimation of phosphate, oxygen and chlorophyll are, by now, routine techniques, and comparatively large numbers of samples can be analysed; (*b*) the mathematical techniques for working up the chemical results seemed particularly applicable to the Fladen ground, which is the main area studied; (*c*) from this method one can hope to obtain, not merely an estimate of production, but also some insight into causes of variations in that production.

Thus, by this approach one can provide quantitative data about standing crop and production which are not obtainable from the older methods of planktology. Alone, however, it provides no detailed insight into the species composition of the populations. Yet it is likely that purely quantitative results may be of most use ultimately in providing connexions between plankton and fishing theory. For example, the effects of variations in the food supply to larval fish and to the bottom fauna might be studied in this way. But before these problems can be understood it is necessary to obtain quantitative knowledge of the causal relations between the phytoplankton and its environment.

This is the aim of this paper, and it will be obvious that it depends on the work which Riley has done in organizing past data and developing this new approach.

The results given here cover 3 years, 1951–53. During the summer and autumn of 1951 general surveys of the Fladen ground were made as part of a herring research programme. Phosphate, temperature and salinity were sampled at 0, 20, 50, 100 m and near-bottom (approximately 140 m), at ten to eighteen stations on five occasions. Two stations were also worked during the first half of the year, one in April and one in June. The analyses of these data suggested that it would be of interest to obtain as often as possible from this area chemical samples with close vertical spacing. Therefore in 1952, at one station on the Fladen ground, phosphate, temperature and salinity were sampled at 10 m intervals on seven occasions between May and November.

Finally, in 1953, the twelve stations shown in Fig. 1 were visited nine times. At each of these stations samples were taken for phosphate, oxygen, salinity, temperature, chlorophyll and dry weight of net hauls. Also at stations 1, 7, 8, 10 and 11 phosphate samples were taken on five other occasions. On most occasions net hauls were taken for species analysis of the plankton.

The first part of this paper uses the phosphate data to provide estimates of production for the years 1951–53. In the second part the 1953 production on Fladen is linked with the chlorophyll and dry-weight data in an attempt to describe the causes of the variations in plant populations throughout the year.

It is a pleasure to thank Dr H. W. Harvey, F.R.S., and Dr G. A. Riley for reading and criticizing this work, although its final form is the author's responsibility.

PART I. PHOSPHATE AND PRODUCTION

The General Hydrographic Conditions

In estimating the utilization of phosphate by the phytoplankton, it is necessary to estimate the changes in phosphate distribution due to water movement. The possible types of change in this area are large-scale lateral movements, i.e. currents, and small-scale movements, i.e. lateral and vertical turbulence. In this paper it is postulated that the only changes which need be taken into account directly in the calculations of production are those due to vertical turbulence. First, the extent to which this postulate holds for the Fladen ground will be considered.

Fladen is the south-west corner of the central area of the northern North Sea lying between the prime meridian and the Norwegian deeps and having a depth of 100–140 m (Fig. 1). During the winter and early spring there are large-scale hydrographic changes in this region due to the inflow of Atlantic water round the north of Shetland. (This can be seen from the surface salinity charts for the North Sea prepared by the Conseil Permanent International pour l'Exploration de la Mer, 1933.) After March, however, the major changes take place in the uppermost layers only. This is shown by the fact that from April to September of any year the bottom temperatures, 6·0–7·5° C, within the dotted area (Fig. 1) remain approximately constant at a value lower than that of any of the surrounding water masses. Since temperature is also constant from the foot of the thermocline (30–70 m) to the bottom, it follows that most if not all of the water below the thermocline which has this lower temperature during the summer must have been in this area since the spring. This water mass forms a cold, and therefore heavy core in the centre of the northern North Sea; the conditions on Fladen are typical in this respect. The possibility of changes within this core, either as circulation or as shifts of position, will be considered when the 3 years' results are discussed in detail.

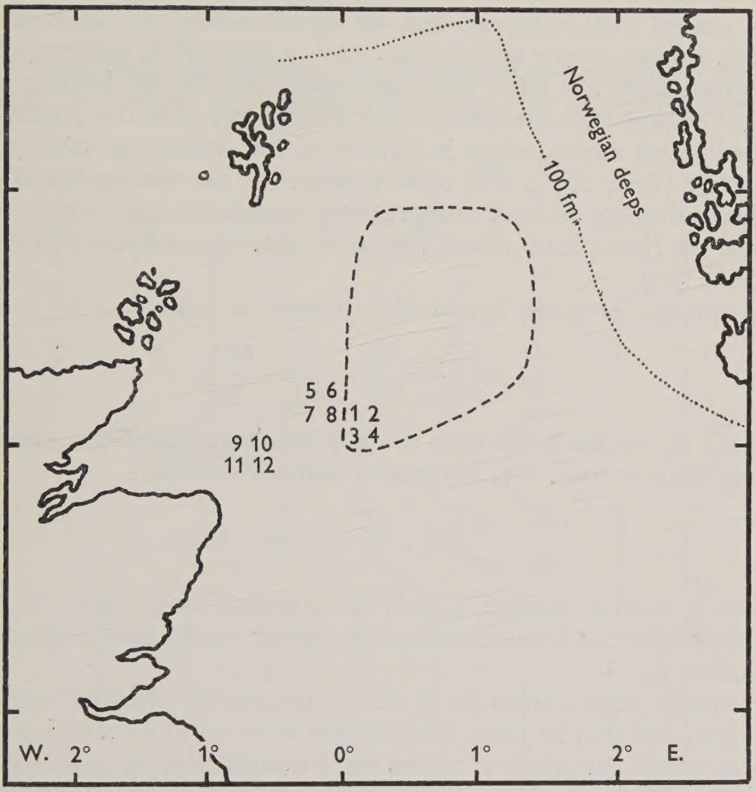

Fig. 1. Positions of stations worked in 1953 in the northern North Sea. 1–4: Fladen Group. 5–8: Northern Group. 9–12: Inshore Group. The dotted line indicates roughly the area inside which there is a nearly constant bottom temperature throughout the spring and summer.

In the surface layers above this cold core, however, large salinity changes occur during the summer and early autumn. These are produced by the westward spread of low salinity water from the current which issues from the Baltic and flows up the Norwegian coast. From the results of drift-bottle experiments, Tait (1937) has inferred that the surface waters of the northern North Sea circulate as an anti-clockwise eddy which occupies nearly the whole of this central area; it appears that the speed of this circulation generally decreases between spring and autumn. It is in this period, however, that the main spread of the less saline water takes place and this may be due partly to increased outflow from the Baltic, but it seems likely to be due largely to the increased lateral mixing which is allowed by the developing stability of the thermocline (Parr, 1936). The reasonableness of the assumption of lateral diffusion as the only mode of change may be tested, using the surface salinity charts for the North Sea (Conseil Permanent International pour l'Exploration de la Mer, 1933).

The equation for purely lateral eddy diffusion of salinity, S, is

$$\frac{\partial S}{\partial t}=\frac{\partial}{\partial x}\left(\frac{A_x}{\rho}\frac{\partial S}{\partial x}\right)+\frac{\partial}{\partial y}\left(\frac{A_y}{\rho}\frac{\partial S}{\partial y}\right),$$

where A_x, A_y are the coefficients of eddy diffusivity (Sverdrup, Johnston & Fleming, 1942, p. 159). Or, in terms of finite differences,

$$\frac{\Delta S_0}{\Delta t}=\frac{A}{\rho a^2}(S_1+S_2+S_3+S_4-4S_0),$$

where A_x is taken as equal to $A_y=A$, $\Delta t=1$ month, $a=30$ miles. S_1, S_2, S_3, S_4 are the salinities at four cardinal points distant a miles from a chosen point with salinity S_0.

Accordingly, eight values for A were calculated for the area considered, four in May and four in June. They ranged from $0{\cdot}3$ to $2{\cdot}0\times10^{+7}$ g/cm/sec, which is within the range of values for the coefficient of horizontal eddy diffusivity given by Sverdrup *et al.* 1942, p. 485. Thus lateral diffusion can be taken as a sufficient explanation of the changes in salinity. It is probable that the effects of this diffusion on the phosphate concentration on Fladen will be negligible since the diffusion has occurred across an area having the same characteristics as Fladen. This is shown in the charts of summer phosphate distribution (Johnston, 1953, fig. 5; and 1954, fig. 8).

Evaluation of 'Biological Change' from Phosphate Observations

By far the largest part of the data collected consists of inorganic phosphate observations. These will be used to provide the estimates of production. The profiles of temperature, phosphate and oxygen for a station on Fladen are shown in Fig. 2.

The calculations are based on two equations (Sverdrup *et al.* 1942, p. 159):

$$\frac{\partial p}{\partial t}=\frac{\partial}{\partial z}\left(\frac{A_p}{\rho}\frac{\partial p}{\partial z}\right)-\frac{\partial C'}{\partial t}+\frac{\partial R'}{\partial t},$$

$$\frac{\partial T}{\partial z}=\frac{\partial}{\partial z}\left(\frac{A_t}{\rho}\frac{\partial T}{\partial z}\right),$$

where p = phosphate concentration, C' = consumption of inorganic phosphate by plants, R' = return of inorganic phosphate due to regeneration from

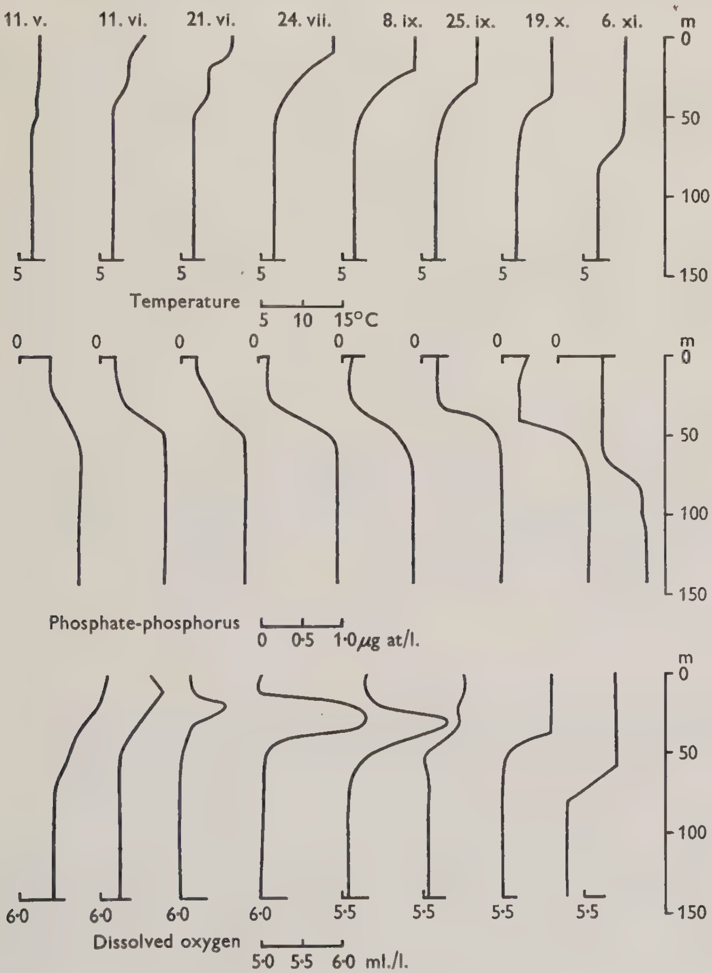

Fig. 2. The profiles of temperature, phosphate-phosphorus and dissolved oxygen at Station 1 during 1953.

organic compounds, T = temperature, z = depth, and A_p, A_t = coefficients of vertical diffusivity of phosphate and temperature respectively.

Integrating both equations from z to 140 m (bottom)

$$\frac{\partial}{\partial t}\int_{140}^{z}(p+C'-R')\,\mathrm{d}z=\left[\frac{A_p}{\rho}\frac{\partial p}{\partial z}\right]_{140}^{z}, \tag{1}$$

$$\frac{\partial}{\partial t}\int_{140}^{z}T\mathrm{d}z=\left[\frac{A_t}{\rho}\frac{\partial T}{\partial z}\right]_{140}^{z}. \tag{2}$$

Taking $$A_p\frac{\partial p}{\partial z}=A_t\frac{\partial T}{\partial z}=0 \text{ at the bottom}$$

and $$A_p=A_t,$$

then substituting (2) in (1) and putting the result in terms of finite differences,

$$\int_{140}^{z}(R-C)\,\mathrm{d}z=\int_{140}^{z}(p_2-p_1)\,\mathrm{d}z-\frac{p'_z}{T'_z}\int_{140}^{z}(T_2-T_1)\,\mathrm{d}z \tag{3}$$

where for any depth p_1, T_1 and p_2, T_2 are the phosphate and temperature values at the beginning and end of a time interval Δt respectively; $C=C'_2-C'_1$, $R=R'_2-R'_1$ are the consumption and return during the time interval Δt, and p'_z, T'_z are calculated as follow:

$$p'_z=p_{z+10}-p_{z-10},$$
$$T'_z=T_{z+10}-T_{z-10},$$

i.e. they are the means of the difference between the phosphate or temperature values 10 m below and 10 m above the depth z.

The integrals are calculated by the usual step-by-step process.

The occasional small variation in the temperature of the homogeneous lower layer is removed by subtracting from the temperature integral

$$I_h=\int_{140}^{z_h}(T_2-T_1)\,\mathrm{d}z,$$

where z_h = depth of the bottom of the thermocline.

Finally, by subtraction, one obtains a set of values for

$$\int_{z+10}^{z}(R-C)\,\mathrm{d}z,\quad z=0, 10, 20, \ldots, z_h. \tag{4}$$

By this method the effect of water mixing on the phosphate distribution is removed and one obtains the change in phosphate content, due to biological activity, in each 10 m layer, during the time interval between two visits to the station.[1]

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[1] Because equation (3) is an approximation, errors will be introduced whose magnitude will depend on the size of the changes in T, p, $\partial T/\partial z$ and $\partial p/\partial z$ between two stations. These are usually small so long as the time interval is not too large.

In practice, because of the low values of the gradients at 10 m, it is usually necessary to take the 0–10 and 10–20 m layers together. Also, of course, the layer from the depth z_h to the bottom cannot be subdivided.

A typical calculation is shown in Table I.

TABLE I. CALCULATION OF 'BIOLOGICAL CHANGE' IN 10 M LAYERS FOR 11–21 JUNE 1953 AT 58° 25′ N., 0° 20′ E.

m	$\int_{140}^{z}(T_2-T_1)\,dz$	p'_z	T'_z	$^{*}\frac{pz'}{Tz'}\int_{140}^{z}(T_2-T_1)\,dz$	$\int_{140}^{z}(p_2-p_1)\,dz$	$\int_{140}^{z}(R-C)\,dz$	$\int_{z+10}^{z}(R-C)\,dz$
0	—	—	—	—	0·61	0·61	
20	8·4	−0·110	1·78	−0·05	0·58	0·63	−0·02
30	9·6	−0·285	1·07	−0·25	0·50	0·75	−0·12
40	5·4	−0·525	1·84	−0·15	0·54	0·69	+0·06
50	0·4	−0·295	0·90	−0·01	0·56	0·57	+0·12
60	0·2	−0·015	0·03	−0·01	0·48	0·49	+0·08
70	0·1	−0·020	0·04	0	0·41	0·41	+0·08
140	0	—	—	0	0	0	+0·41

* To convert to μg at/cm² the figures have been multiplied by 10^{-1}.

Analysis of Phosphate Observations

If the figures given by (4) are to be used to provide estimates of production, then it is necessary to make hypotheses about the ways in which consumption and return of inorganic phosphate occur in the sea.

As expected, the values of (4) show a decrease in the upper and an increase in the lower layers, with the change-over occurring at about 30–40 m. This depth of zero change will be called the 'zero level'.

Thus the simplest assumptions would be: (*a*) only production occurs above the zero level and only regeneration below; (*b*) the production in terms of carbon can be found by using a fixed carbon-phosphorus ratio (Sverdrup *et al.* 1942, p. 237).

The objections to these postulates must be considered; the main ones appear to be as follows:

(i) There will be some regeneration above the zero level. This regeneration may occur at a rate equal to the mean rate of regeneration below the zero level, or it may be much greater since there is more phytoplankton above the zero level than below it. The factors involved in regeneration are complex. Since the plants in the euphotic zone are assumed to be living and will not normally die until they sink below the euphotic zone it may be that regeneration from direct decay of plants will be very small. Whether or not regeneration from zooplankton metabolism will be equally spread throughout the water column is at present unknown. Thus no good estimate can be given for the effect of regeneration of phosphate above the zero level. In the conclusion to this part of the paper an estimate is made of the effect of assuming equal regeneration at all depths, but in the body of the paper assumption (*a*) has been used as being the simplest method of stating the results.

(ii) There is evidence that plants can use organic, as well as inorganic, phosphorus compounds to meet their phosphate needs (Chu, 1946; Harvey, 1953). This is an unknown factor to the extent that this evidence comes from laboratory experiments and the process has not been demonstrated under natural conditions. (It has also been suggested (Lwoff, 1951) that some flagellates may be able to ingest particles of organic matter. This, however, does not affect estimates of basic production, which are in terms of the conversion of carbon from inorganic to organic form.)

(iii) The most serious objections to the second postulate comes from laboratory work on phosphate uptake of phytoplankton. Ketchum (1939) showed that in a reduced phosphate concentration ($< 0{\cdot}55$ μg at/l.) diatoms can continue to photosynthesize carbon without a corresponding uptake of phosphate. The phosphate concentration near the surface in the North Sea is less than 0·55 μg at/l. throughout most of the summer. Ketchum showed that cells in a deficient medium could double in number without the uptake of phosphate. To discover the possible effects of this, consider the position at the end of the spring outburst on Fladen. On 11 June 1953 the population in the euphotic zone, as estimated from the chlorophyll content, contained 2·4 g carbon/m^2. If this doubled itself without consuming phosphate then the increase in production would be 2·4 g carbon/m^2. But this is only 5% of the year's production. Further, before this deficient population can again divide, there must be replacement of the phosphate deficiency, and this will be shown by the phosphate changes. For these reasons, the effect of phosphate deficiency may not be appreciable.

These objections must be borne in mind when the validity is considered of the following estimates as absolute measures of production. However, they should not seriously affect conclusions about variations in production from year to year or place to place, and such results are among the main aims of this paper.

Production on Fladen

For 1951, the temperature and phosphate values were obtained by choosing from extensive surveys those stations near the centre of the Fladen Ground that showed similar structure (the stations on the south and west edges of Fladen showed greater mixing and, often, salinity changes). From the average of the chosen stations the changes above and below 35 m were calculated, and the estimated production is given as a histogram (Fig. 3A). These values will not be particularly accurate due to the small number of depths at which chemical observations were made; thus it is not possible to study the data in more detail.

For 1952, the production is again given as a histogram (Fig. 4A), while the profiles for each interval are shown in Fig. 6.

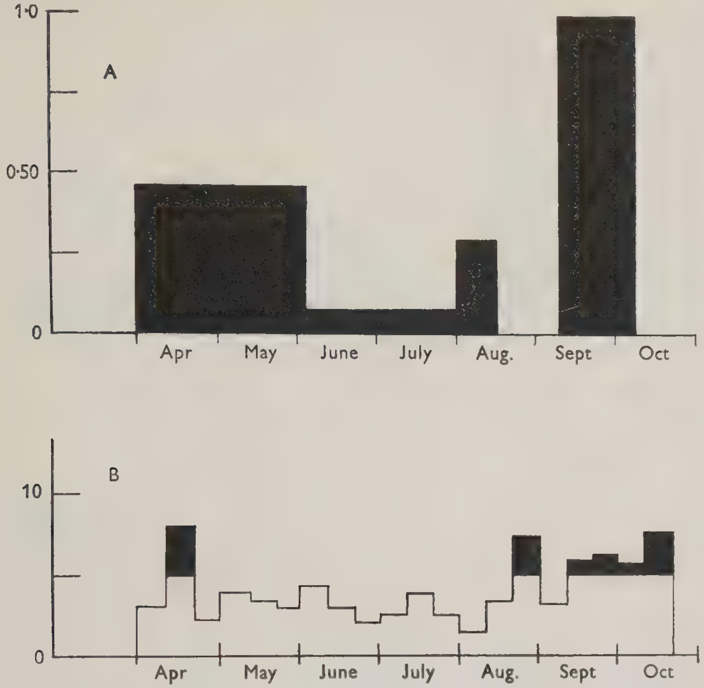

Fig. 3. 1951: A, production rate on Fladen, g carbon/m^2 day; B, wind velocity, m/sec.

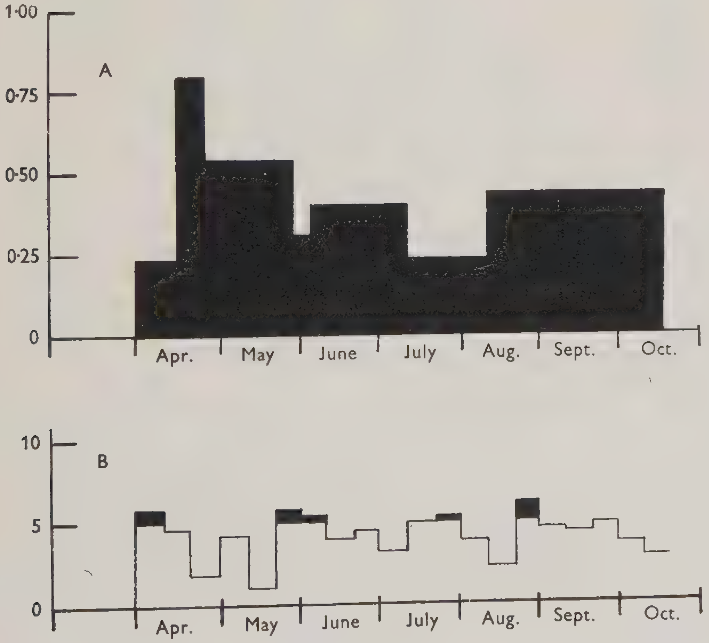

Fig. 4. 1952: A, production rate on Fladen, g carbon/m^2 day; B, wind velocity, m/sec.

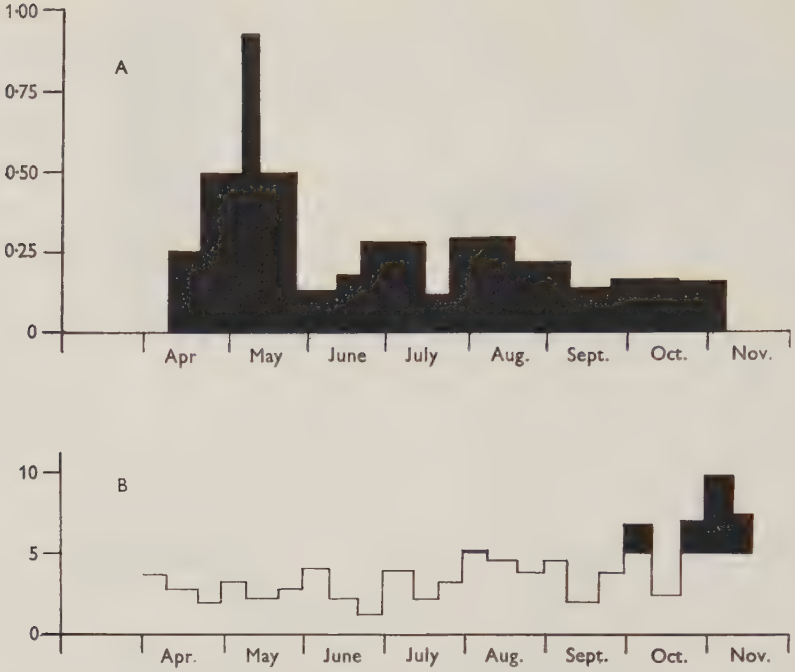

Fig. 5. 1953: A, production rate on Fladen, g carbon/m^2 day; B, wind velocity, m/sec.

The histogram for 1953 (Fig. 5A) is also constructed from the mean values for production between the main surveys, but detail is added by using the intermediate observations at station 1 to give the relative ratio of production in the adjacent parts of the main interval. The profiles in Fig. 7 are from the means for the main surveys, with the exception of the last which is from station 1. For reference the dates of sampling are given in Table II.

At this point some of the practical difficulties should be mentioned which arise in working up the data. First, there is the occasional aberrant observation, for which a corrected value is taken by interpolation from a graph (e.g. from a phosphate-depth profile). Also, on two occasions, all values at one station differed considerably from the preceding and following stations. At station 1 on 15 July the salinities were very low, and on 18 August the phosphates were very low. These may be due to a faulty standardization and have been omitted.

Secondly, more important difficulties arise when $T_2 < T_1$ at depths on the lower part of the thermocline. This would give an apparent 'negative heat transport', which is physically impossible. Apart from the usual errors due to wire angle caused by ship drift, etc., the most likely cause of this anomaly will be internal waves. In the calculations the effect has been removed by taking the second temperature equal to the first and adjusting the phosphate proportionately. These corrected values are also used for the 'T_1' and 'p_1' in the next interval. This process will leave in doubt the values of production for the two separate intervals, but should not affect the value of their sum as the total estimate for the two intervals taken together.

However, once such inaccuracies have been allowed in a few particular cases, it must be admitted that they may occur in others without their presence being noticed (i.e. without $T_2 < T_1$). Until detailed knowledge is available of the variations due to internal waves, the limits of these errors cannot be given.

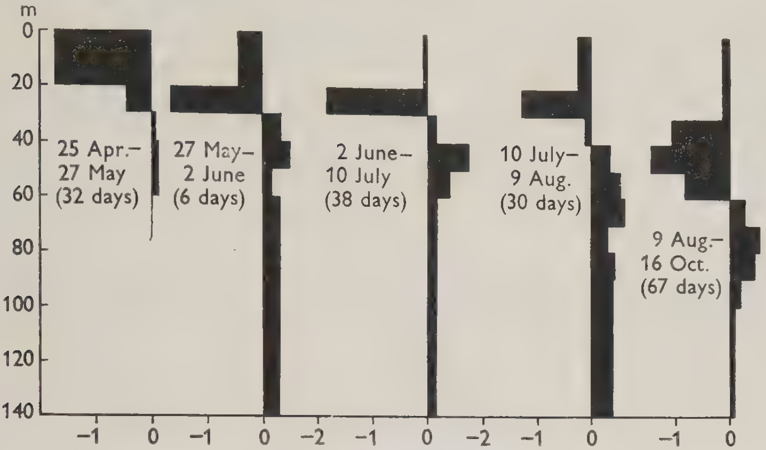

Fig. 6 1952: profiles of 'biological change' in phosphate on Fladen in $10^2 \times \mu g$ at./cm^2 day

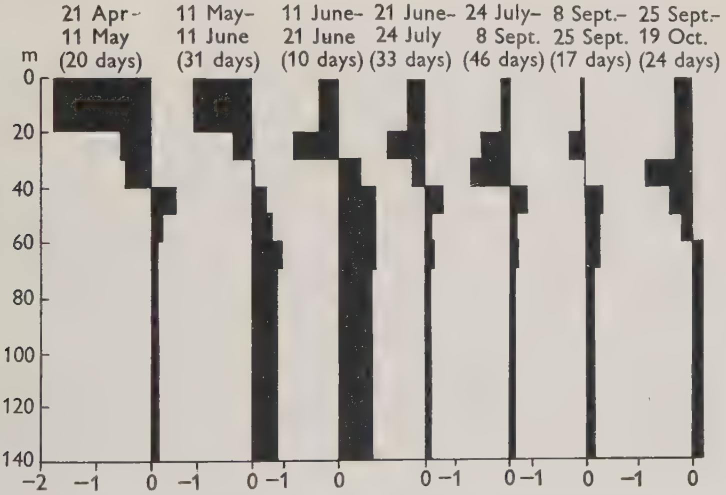

Fig. 7. 1953: profiles of 'biological change' in phosphate on Fladen in $10^2 \times \mu g$ at./cm^2 day

TABLE II. DATES OF SAMPLING

1951	2. iv, 3. vi, 30. vii, 16. viii, 10. ix, 7. x
1952	15. iv, 25. iv, 27. v, 2. vi, 10. vii, 9. viii, 16. x
1953	5. iv, 21. iv*, 5. v, 11. v*, 26. v, 11. vi*, 21. vi*, 15. vii, 24. vii*, 18. viii, 8. ix*, 25. ix, 19. x, 6. xi

* Main surveys.

The profiles will be discussed first. Until the beginning of June they give the conventional picture of maximum production in the upper 20 m. From June onwards, maximum decrease in phosphate occurs below 20 m. This is found in both 1952 and 1953 (and at all four stations in 1953). Also, during the summer, the level of maximum decrease falls from 20–30 to 30–40 m. (The profile for 9 August to 16 October 1952 is likely to be inaccurate due to the length of the interval, 67 days, and the fact that there were large changes of temperature.) The interpretation of this feature is difficult. It could correspond to genuine photosynthetic activity, or it might be due to dark uptake of phosphate by deficient plants (Ketchum, 1939) on sinking into the top of the phosphate 'cline'. However, a study of oxygen[1] profiles (Fig. 2), shows that there was an increase in oxygen concentration at 20 and then 30 m during the summer. This implies that photosynthesis was occurring at these depths, and it will be assumed in Part II of this paper that the phosphate changes correspond to plant production.

TABLE III. FLADEN PRODUCTION (G CARBON/M^2)

	Spring	Summer	Autumn	Total
1951	28·0	8·9	28·0	64·9
1952	30·4	22·4	29·5	82·3
1953	26·1	17·6	13·7	57·4

The production estimates will be divided into three parts (Table III): the period until the first half of June corresponding to the spring outburst; that from the middle of June until the middle of August to the summer production; and from then until about the middle of October to the autumn flowering. After the middle of October signs appear of the start of the large-scale hydrographic changes mentioned earlier. Estimates for October–November production are erratic, but it is unlikely that the production will have been very great: a value is given for this period in 1953, but in 1952 the large salinity changes made such an attempt useless.

For the spring outburst there is a variation of only 14% between the 3 years. This fits with the conventional picture of a spring outburst following the establishment of the thermocline and limited by the amount of nutrient above this thermocline.

In the summer and autumn there are more than twofold variations in the production. The variations in the summer production will depend on three factors: (i) a general reduction in phosphate concentration in the euphotic zone from the June level of approximately 0·25 μg at/l. down to values below 0·10 μg at/l. (this may correspond with the ability of dinoflagellates to assimilate phosphate at lower concentrations than the spring forms—Barker, 1935; Braarud & Rossavik, 1951); (ii) an increase in the depth at which maximum consumption occurs; (iii) the consumption of any phosphate carried upwards by mixing.

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[1] The analysis of the oxygen data involves discussion of several difficulties, and it is intended to use these data in conjunction with future and more detailed work in further attempts to understand these problems.

To show that only the third of these factors varies considerably, the figures for phosphate decrease are separated into their components in Table IV. $\int p_2 - p_1$ = changes above the zero level due to (i) and (ii). I_p = upwards transport through the zero level corresponding to (iii). The net decrease is given by $\int (p_2 - p_1) - I_p$, in μg at /cm.^{2}.

TABLE IV. TRANSPORT THROUGH, AND CHANGE ABOVE, THE ZERO LEVEL

1952	$\int p_2 - p_1$	I_p	1953	$\int p_2 - p_1$	I_p
2. vi–10. vii	−0·71	0·44	11. vi–21. vi	0·07	0·15
10. vii–9. viii	−0·04	0·51	21. vi–24. vii	−0·67	0·03
9. viii–16. x	+0·12	1·92	24. vii–8. ix	0	0·73
			8. ix–25. ix	+0·18	0·27
			25. ix–19. x	−0·20	0·20
Total	−0·63	2·87		−0·62	1·38

Further, in 1951, when there was a very steep thermocline and apparently little transport, the summer decrease is 0·70 μg at/cm.^{2}. This value, with those of 0·75 and 0·60 for 1952 and 1953, shows that the variation in the summer due to the first two factors is comparatively small. This basic summer production is estimated at 8·7 g carbon/m^2 and has its effect at the end of June and the beginning of July. After that, production is solely dependent on the upwards transport of phosphate.

Thus, in the autumn, production depends on the breakdown of the thermocline. This occurred during September in 1951 and 1952, but in 1953 did not take place until after the middle of October, and it appears that owing to this delay the autumn production was less than half that of the previous year.

These variations in transport can best be shown in terms of the heat transported downwards through 40 m. Fig. 8 illustrates the differences between the three years. In a region such as Fladen, which is not subject to strong currents, vertical mixing must be almost entirely due to wind. Below each of the histograms of production is shown the corresponding wind data (Figs. 3B, 4B, 5B) for the northern North Sea (supplied by courtesy of the German Hydrographic Institute, Hamburg, through the Conseil Permanent International pour l'Exploration de la Mer). These are in the form of 10-day means calculated from atmospheric pressure gradients. The exact effect of strong winds on the thermocline is not known, especially in relation to the time factor. Thus it is not possible to say whether 10-day means should be a reliable guide in comparing wind and thermocline changes. However, the main variations noted in mixing can be related to the wind histograms.

The greater amount of summer mixing in 1952 than in the other 2 years was due to the fact that the thermocline, which had half formed by the end of May, was partially broken down at the beginning of June with the result that, for the remainder of the year, the thermocline was not as steep as in 1951 and 1953. From the wind histograms it can be seen that only in 1952 were there mean winds in June exceeding 5 m/sec.

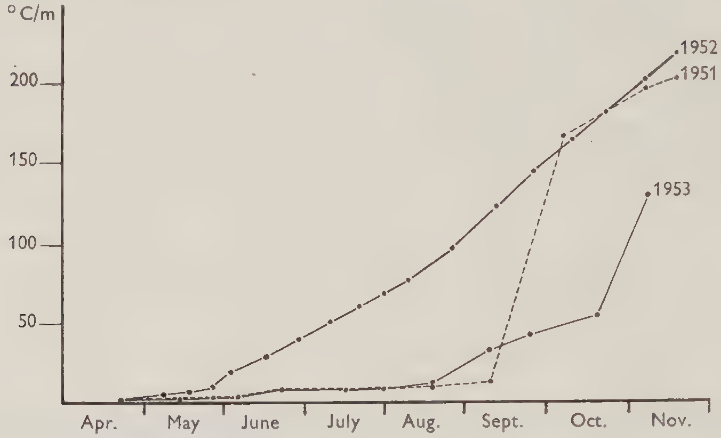

Fig. 8. Increase in the heat content below 40 m on Fladen.

Since it is reasonable to suppose that the strength of wind required to break down the thermocline is roughly inversely proportional to the steepness of the thermocline then, unless the thermocline is partly broken during early June, it is less likely to be broken throughout the remainder of the summer. For this reason, gales at the beginning of June should be critical in determining the excess of summer production over the basic amount previously mentioned.

During the first 10 days of August 1953 there were winds in excess of 5 m/sec which may have caused the transport noted for August. However, from then until October there are no signs of strong winds, and this feature corresponds to the delayed and consequently small production in the autumn, compared with 1951 and 1952 when there were winds greater than 5 m/sec.

Thus the expected chain of relations, strong winds—mixing—production, can be seen to operate on Fladen.

Phosphate Regeneration on Fladen

The estimates of regeneration (Figs. 9, 10) are extremely variable, both within years and between years. To bring any order into them requires a fair amount of 'inspired guessing' at the reasons for these variations, and so the conclusions reached are, to that extent, speculative.

First, it is necessary to consider the effect of known errors in phosphate observations for June 1953. Analyses of duplicate samples from station 1, 21 June 1953, were carried out at the Plymouth Marine Laboratory. These gave consistently lower values than those from the analyses in this laboratory; also there was an apparently greater scatter in our own results. Shortly afterwards the galvanometer in use in this laboratory broke down completely, and it is probable that the onset of the defect caused this difference in results.

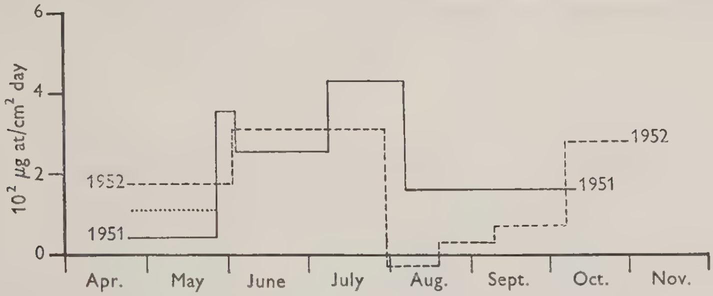

Fig. 9. Regeneration rate of inorganic phosphorus on Fladen in 1951 and 1952. ..., correction made by taking winter phosphate concentration in 1952 as 0·73 μg at/l. (see text). ($10^2 \times$ μg at/cm² day.)

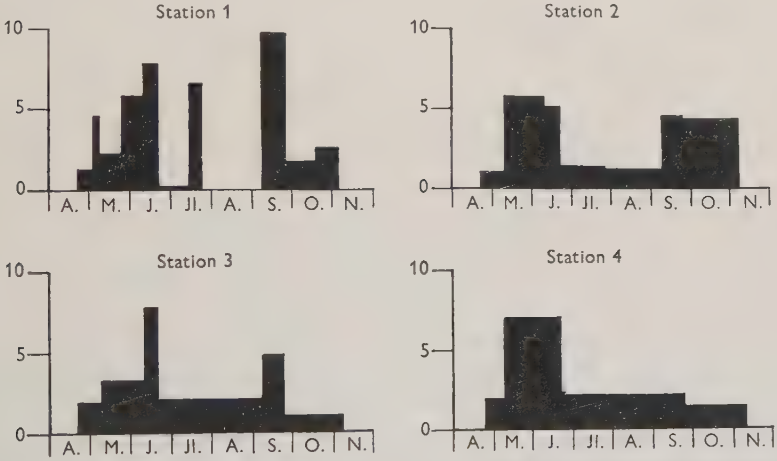

Fig. 10. Regeneration rate of inorganic phosphorus at the four Fladen stations in 1953 (in $10^2 \times$ μg at/cm² day).

In Table V the effects of these differences are given. The effect on production is less than 10% and nearly balances over the two intervals. But, although regeneration also nearly balances, the internal differences are very large. This is due to the fact that regeneration is very sensitive to the absolute value of the phosphate concentration, whereas production depends more on the phosphate gradient. This example is of interest in showing the effect of inaccuracies on the phosphate observations, and because of this, no alterations were made in the production estimates. The data have been used to correct both the 1953 estimates for station 1 and those for the other stations in the Fladen Group, and explains one of the differences between the histograms of regeneration for 1951, 1952 (Fig. 9) and 1953 (Fig. 10).

TABLE V. COMPARISON OF PHOSPHATE ESTIMATES (μg AT/CM^2)

	Aberdeen		Plymouth	
	Production	Regeneration	Production	Regeneration
11. v.–21. vi	0·82	2·02	0·91	1·22
21. vi–15. vii	0·62	0·01	0·55	0·83

The next anomalous feature of the 1953 results is the large rate of regeneration found in the period 8–25 September. This is due to the large increase in the phosphate concentration in the lower isothermal part of the water column. However, there is also an increase in salinity at the two northerly stations of the Fladen group (Fig. 11). Now, inside the central area of the northern North Sea described on pp. 2–4, although the temperature is relatively constant, there are variations in salinity and phosphate concentration due to the unequal mixing of the winter Atlantic inflow. It is suggested that during September there was a shift in the central core, bringing water of higher phosphate concentration on to Fladen. This shift could be due to the 'pressure' begun by another Atlantic inflow. Fraser (1954), from plankton distributions, and Tait (1954) show that during the summer there was a surge of oceanic water round the north of Shetland, and that during the autumn this water penetrated into the North Sea till, by the end of the year, it covered the Fladen Ground. The plankton collections taken on Fladen agree with this general picture, which thus provides a reasonable explanation for the shifting water mass.

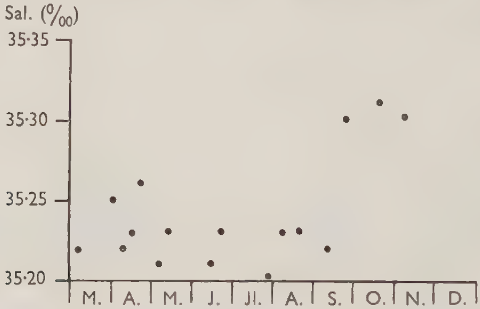

Fig. 11. Salinity at 58° 25′ N., 0° 20′ E. (Fladen) in 1953; an example of the changes which occurred in September.

A further demonstration of the anomalous nature of this feature is shown in the graphs of integrated content of phosphate in the water column (Fig. 12), where the graph for 1953 suddenly rises much above the content at the beginning of the season. This anomaly has been removed by using the mean rate of regeneration of the surrounding intervals.

Again, from Fig. 12, it is possible to notice another anomaly. In 1952 the spring regeneration was much lower than in the other 2 years. Now the winter phosphate concentration is obtained from three phosphate values, 0·73, 0·76, 0·79 μg at/l., in the lower part of the water column on 25 April; the mean 0·76 μg at/l. was chosen, but in Fig. 12 this is seen to put the winter level abnormally high compared with the remainder of the curve (even though there was more production in this year than in the other two). A reduction in the winter level to 0·73 μg at/l. would raise the estimate of spring regeneration from 0·32 μg at/cm² to 0·56 μg at/l.; at the same time the reduction in the spring production would be 1·7 g carbon/m², bringing the total down to 28·7 g carbon/m², which is in fact nearer to the other two estimates.

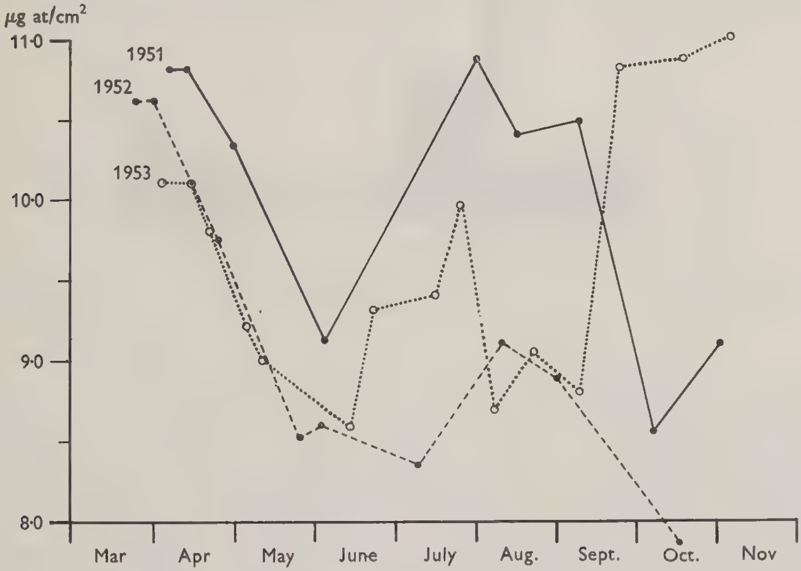

Fig. 12. Integrated content of inorganic phosphorus on Fladen in the years 1951–3 (μg at/cm²).

The results of these various corrections are shown in Figs. 9 and 13, which are the histograms of regeneration for Fladen. They show that the main regeneration after the spring outburst occurs between the middle of June and end of July. Thus, the maximum rate of regeneration occurs about 2 months after the maximum rate of production, and this interval also corresponds to the interval between the end of the spring outburst (end of May) and the beginning (end of July) of the low rate of regeneration found in the summer of 1951 and 1953.

From this it appears that regeneration occurs about 2 months after production. Again, this effect can be seen from the graphs of Fig. 12, where the interval between the spring trough and summer peak is approximately 2 months, and the phosphate content at this summer peak in 1951 and 1953 is about the same as the winter level.

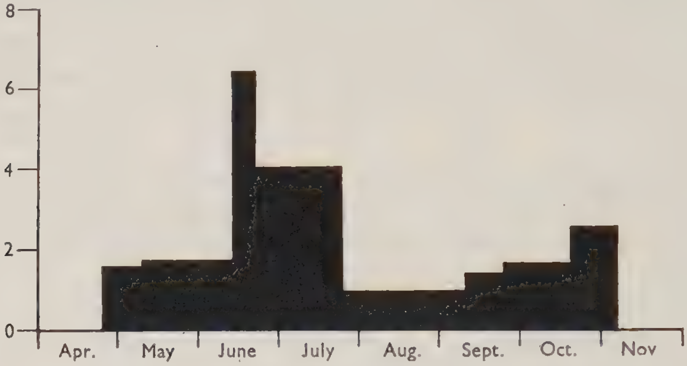

Fig. 13. Corrected regeneration rate of inorganic phosphorus on Fladen in 1953 ($10^2 \times \mu$g at/cm^2 day).

Production in the Northern and Inshore groups

The northern group of stations (Fig. 1) shows the transition from the conditions on Fladen to a regime of greater mixing. This is illustrated by the vertical heat transport at the stations in 1953 (Fig. 14). Stations 6, 8 and 7 show increased mixing in that order, ending, however, at approximately the same total in November. Station 5 is very erratic; the bottom temperature does not show any regular increase, and it is probable that the lateral gradient in the amount of mixing is sufficiently steep for these changes to be due to small horizontal shifts in the water between times of sampling. The temperature profiles for station 7 are given in Fig. 15A.

Calculations for production were made in the same way as for Fladen, although the postulate of purely vertical water movement may not always be satisfactory.

During the summer of 1953, the greater mixing gave rise to an increased production which probably equals that of the summer production on Fladen in 1952 (the maximum on Fladen in the three years considered).

In the autumn of 1953, there is much larger production than on Fladen. An explanation of this may be found in Fig. 14. The heat transport by 6 November is much higher than on Fladen 1953 and is almost the same as that found on Fladen in 1951 and 1952. Thus it appears that the breakdown of the thermocline occurred earlier than on Fladen. This is confirmed by the production figures for station 7, where it is shown that the main part of the autumn flowering occurred before 19 October. On Fladen (station 1) there was but little production before that date in 1953.

Thus the northern group, which is in an area of hydrographic change between the conditions on Fladen and those inshore, shows corresponding changes in the production.

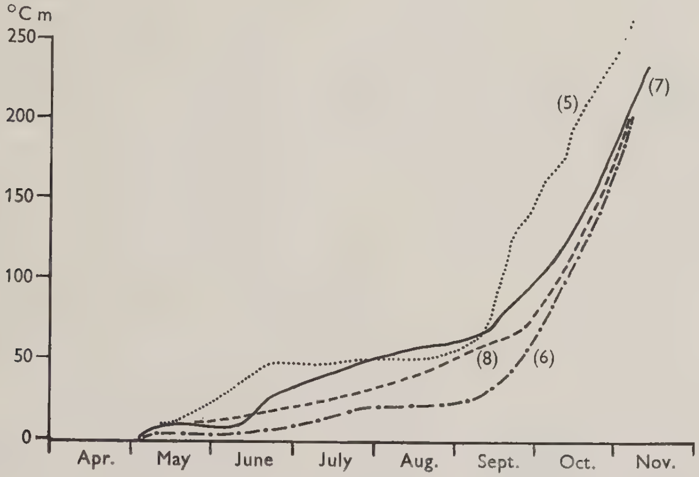

Fig. 14. Increase in heat content below 40 m at the Northern group of stations in 1953.

TABLE VI. 1953: PRODUCTION IN NORTHERN GROUP (G CARBON/M^2)

Station no.	15. iv–11. vi	11. vi–8. ix	8. ix–25. ix	25. ix–19. x	19. x–6. xi	Total
5	24·2	20·5	8·3	27·0		80·0
6	23·2	18·7	5·7	20·9		68·5
7	23·7	36·5	19·0		7·1	86·3
8	28·9	25·7	4·3	11·6		70·5

The inshore group of stations lies in an area of confused water movement with large changes in salinity. From an examination of the salinities it seems likely that most of the time these changes are due to local wind drift which will produce variations in the seawards limit of the less saline coastal water. However, there will be occasions when the changes are not local, but will involve the intrusion of water from other regions. The temperature profiles for station 11 are given in Fig. 15B.

Thus it is obvious that the postulate of purely vertical water movement does not hold, yet, since these lateral changes may be of an oscillatory nature for much of the year, the total estimates of production, considered as a balance of these variations may give a reasonable estimate for the year.

The values of yearly production for the four stations were 103·9, 108·1, 108·4 and 127·2 g carbon/m².

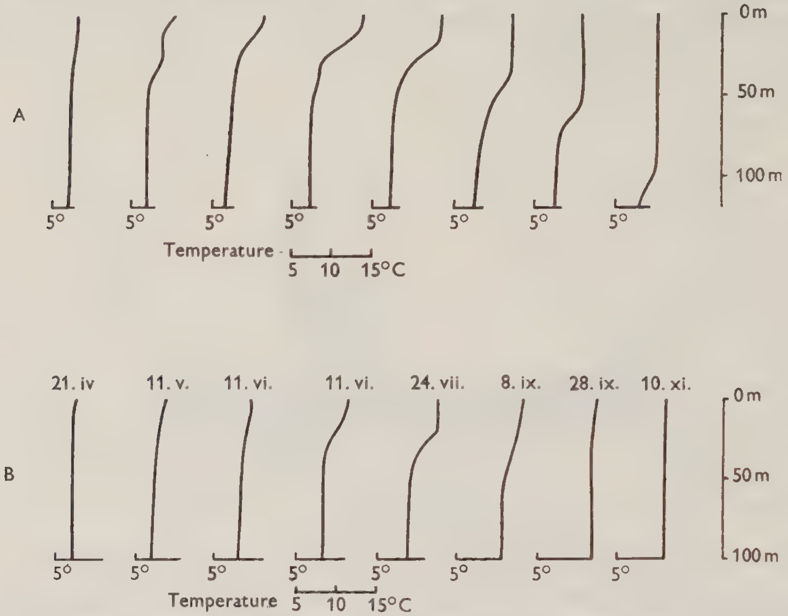

Fig. 15. The profiles of temperature, at A a station in the Northern group, and B a station in the Inshore group.

Table VII. Summary of the Estimates of Production (g carbon/m²)

Group	1951	1952	1953				
			Station	1	2	3	4
Fladen	64·9	82·3		59·5	53·6	56·3	62·2
			Station	5	6	7	8
Northern	—	—		79·9	68·4	86·2	70·5
			Station	9	10	11	12
Inshore	—	—		127·2	103·9	108·1	108·4

Discussion

The values for yearly production given in Table VII show variations between years on Fladen and between groups in the year 1953. The main conclusion of this part is that the differences in the Fladen results can be explained by the variations in mixing, and these variations in turn can be linked with the wind data and show the critical effects of high winds near the beginning of June and at the middle of September.

Fladen was chosen as typical of those parts of the North Sea where least mixing occurs. The other groups were added to show the effects of increased mixing and in their values for production they reflect this. Thus in 1953 the inshore group had a production nearly twice that of the Fladen group, while the northern stations had intermediate values.

This increased mixing will be largely due to the increase in the effect of tidal movement. On Fladen the wind effectively controls mixing, and it was possible to relate the two. Such relations would not be demonstrated in the more complex cases when wind and tide combine.

As was stated earlier (p. 7) the effect of regeneration above the zero level has been ignored. In Figs. 6 and 7 the depth distribution of regeneration below the zero level is shown. Although there are variations with depths, there is no sign of a consistent increase in the rate of regeneration just below the zero level. Thus the assumption that the regeneration rate is constant with depth may be satisfactory. On this basis the addition to the values of production already given would be about 17 g carbon/m^2 on the Fladen and Northern groups and about 31 g at the Inshore group. This is an increase of between a quarter and a third of the original estimates.

TABLE VIII. COMPARISON OF NORTHERN NORTH SEA, ENGLISH CHANNEL AND GEORGES BANK

	Winter phosphate (g at/l.)	Yearly production (g. carbon/m^2)	Maximum plant population (mg chlorophyll/m^2)	Maximum zooplankton (g carbon/m^2)
Fladen	0·7	54–82	100	5
Inshore	0·6	104–127	175	5
English Channel	0·35	55–91	210	5
Georges Bank	1·1	120–300	660	30

These estimates of production can be compared with those from other areas. It is pointless to compare production values by themselves, so three other relevant figures are given in Table VIII. It will be seen that the English Channel (station E. 1), although having a lower winter phosphate concentration than the northern North Sea, has a production which is probably between Fladen and Inshore values. This is due to the fact that there is at E. 1 vertical mixing similar to that occurring at the Inshore group. Georges Bank, which has both a large winter phosphate concentration and considerable mixing, gives a production which is certainly greater than any of the other regions, and these differences are reflected in the maximum plant and zooplankton populations.

Thus the estimates of production given in this paper fit reasonably into the pattern of previous results. Because of this, and because of the internal consistency of the Fladen results, it seems that the use of phosphate data can be considered as a comparatively satisfactory method of studying production.

PART II. PLANT POPULATION AND PRODUCTION

The first part of this paper was mainly concerned with the problem of estimating the production from the sea area typified by Fladen. The various assumptions required for these estimates were each more or less open to question, and thus the results are to that extent uncertain.

In this second part, these results will be linked with chlorophyll and dry-weight data, again on the basis of hypotheses whose validity is uncertain. The picture obtained, however, proves to be to some extent internally consistent and agrees reasonably with the results of other workers. To this extent the various methods of sampling and their attendant hypotheses can be held to be justified.

The attempts to describe quantitatively the causal relationships which control plant population and production began with the work of Fleming (1939) and were continued by Riley and his colleagues (Riley, 1946; Riley *et al.* 1949). The development of this approach is summarized by Riley (1953).

Any model of the plant population should take account of many factors, but there are three principal ones which must be considered: production, predation and water movement. Fleming (1939) and Riley (1946) did not deal with the effects of vertical mixing, and thus said nothing about the vertical distribution of the plants. Instead they concentrated on changes with time and obtained reasonable fits with the mean populations. The problem of explaining the distributions with depth was studied by Riley *et al.* (1949) but, in order to do this, areas and times of year were chosen in which the plankton was changing slowly and this was idealized by assuming a steady state. Again fairly good fits were obtained.

Thus the present position is that changes in mean population with time, and stationary distributions with depth, have been studied separately, but no attempts have yet been made to provide an example where these two factors occur together. The main obstacle to this is caused by the generally continuous lateral changes which occur in the sea. These ensure that the water sampled on a given date will frequently not be the same as that sampled previously. This is most serious when detailed vertical distributions are being studied.

However, it was shown in the first part of this paper that the Fladen area appears to approximate sufficiently to the required conditions. For this reason an attempt will be made to compare calculated and observed distributions of chlorophyll throughout 1953.

Ideally one should start from measurements of the physical environment alone and, by means of a set of simultaneous equations, build up models of the distribution of nutrients, plants, herbivores, etc. This will not be attempted here, and only the distribution of the plants as shown by chlorophyll concentration will be considered.

Also, there should be a genuine prediction which can be tested, but this is impossible since, in the end, wind strength is unpredictable. Thus to explain the chlorophyll distribution at the end of a time interval, the information used is as follows: chlorophyll distribution at the beginning of the interval, phosphate, temperature and dry-weight of a net haul at both ends of the interval. In this way, for a series of intervals, a series of calculated chlorophyll distribution is obtained for comparison with the observed distributions.

Before giving the calculations, the chlorophyll and dry-weight observations will be described.

In 1953, when it was decided to supplement the chemical observations by estimates of the chlorophyll concentration, the following simple method was devised.

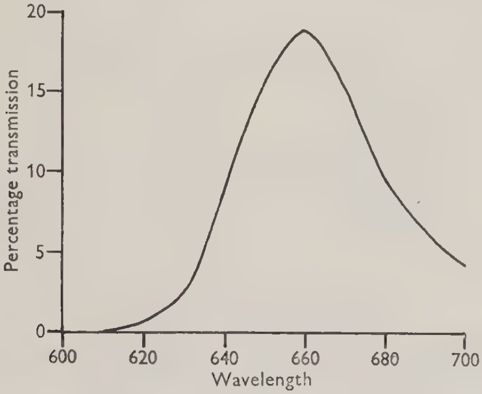

Fig. 16. Calculated spectral response of combination of photocell and I 608 filter.

The Chlorophyll Observations

Water samples were taken from 10, 30, 50 and 100 m. The quantity of water used for each estimation was either 1·6 or 2·4 l. This was filtered under suction through a no. 542 Whatman filter-paper and the paper preserved in 80% acetone. The sample was kept in the dark until it was taken to the laboratory where it was made up to 25 ml. after filtering and its light absorption measured. The measurement was made on the long cell model of the Hilger and Watts 'Spekker', using a 3 in. cell and Ilford 608 filters. The spectral response given by the combination of the filter and the cut-off of the photoelectric cell is shown in Fig. 16. Since the secondary chlorophyll maximum, which is used in this type of work, lies at about 655 mμ, the response curve used should be satisfactory. The main drawbacks are (*a*) less than half of the light beam is used, and (*b*) the maximum transmission of 19% is low.

The instrument was calibrated with a known chlorophyll concentration kindly provided by Dr de Kock of the Macaulay Institute for Soil Research, Aberdeen.

One source of error which should be mentioned is the possible loss of the smallest plants, which may pass through the filter-paper. Using a no. 2 Whatman paper, Riley (1941) concluded that he caught 90% of the phytoplankton, a membrane filter catching little more. Harvey (1950), however, found that a no. 2 paper caught just over half of what was retained on a Gradocol membrane. The no. 542 paper used here is probably more retentive than the no. 2, but the probability of the small flagellates escaping cannot be ignored.

Thus this arrangement cannot be expected to be very accurate. However, apart from inaccuracies in the method, one can expect significant and perhaps large errors to arise from sampling variations.

TABLE IX. REPEATED SAMPLING, 58° 15′ N., 0° 20′ E.—21 JUNE 1953

(Temperature ° C; chlorophyll mg/m^3.)

10 m	Temperature	11·47	11·50	11·52
	Chlorophyll	0·4	0·2	0·4
30 m	Temperature	8·49	8·49	8·51
	Chlorophyll	1·6	1·0	1·4
50 m	Temperature	8·29	7·34	6·79
	Chlorophyll	1·2	1·0	0·4
100 m	Temperature	6·70	—	—
	Chlorophyll	0·3	—	—

Variation in the Results

On three occasions repeated sampling was carried out and the values are shown in Tables IX and X.

In Table IX are shown the results from a station of the Fladen Group. The temperature of each water sample was also taken. It will be seen that the largest variation in concentration occurs at 50 m where there are large temperature changes. These variations are related; the highest temperature at 50 m is near the 30 m temperature and the chlorophyll concentration lies within the range of the 30 m values; similarly, the lowest temperature at 50 m is near the temperature of the homogeneous lower layer and the corresponding concentrations do not differ significantly. Thus, at this particularly steep point of the thermocline, the variations in concentration depend upon the part of the thermocline from which the sample was taken.

Considering the further results from Table X, it may be said that, when the 50 m case in Table IX is omitted, the standard deviation is not more than 25% of the mean, except at such low concentration as 10 m on 21 June 1953.

The next form of variation to be considered is that between stations of the Fladen group at any given time. All the observations are given in Table XI.

This shows that on Fladen, although there are differences between the four stations on any occasion, the maximum concentrations nearly always occur at the same depth. That is to say, the samples from the four stations appear to be samples of populations having the same distribution with depth. Thus it is reasonable to take the mean values of the four stations, as shown in Fig. 18, as representing the chlorophyll distribution on Fladen.

Table X. Repeated Sampling from the Inshore Group

57° 55′ N., 1° 00′ W., 25. vii. 53	10 m	0·6	—	0·7	—
	30 m	2·1	2·0	2·6	—
58° 05′ N., 1° 00′ W., 8. ix. 53	10 m	1·8	1·5	1·6	1·9

Table XI. Chlorophyll Concentration in mg/m^3 on Fladen in 1953

	21. iv				11. v				11. vi				21. vi			
Station no. ...	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
10 m	0·6	—	1·0	0	2·9	1·9	—	2·9	0·8	0·4	1·2	1·1	0·4	0·4	0·4	1·0
30 m	0	—	0·8	0	1·6	0·7	—	—	1·5	1·3	1·4	1·2	1·4	1·2	1·6	1·7
50 m	0	—	0·6	0	0·2	0·3	—	0·4	0·4	0·4	0·6	1·4	0·8	0·4	1·1	0·7
100 m	0	—	0·2	0	0	0·3	—	0·5	0·2	0·6	0·4	0·6	0·4	0·4	0·3	0·4

	24. vii				8. ix				25. ix			
tation no. ...	1	2	3	4	1	2	3	4	1	2	3	4
10 m	0·2	0·2	0·2	0·1	0·4	0·4	—	0·4	0·7	0·3	0·8	0·9
30 m	0·3	0·4	0·5	0·4	0·4	0·8	—	0·6	0·3	1·0	0·7	1·2
50 m	1·0	1·0	0·6	1·7	0·8	0·8	—	0·7	0	0·2	0·3	0·6
100 m	0·2	0·3	0·6	0·5	0·4	0·4	—	0·1	0·2	0·2	0·2	0·2

Since the chlorophyll observations are to be used in conjunction with phosphate and dry-weight data, some common quantity is required and, as in the phosphate results, carbon content will be used. However, the initial question is whether it is possible to have a single conversion factor from chlorophyll to carbon content. It has been shown that the concentration of pigments in plant cells varies with the light intensity in which they are grown (Rabinowitch, 1945). Similar variations have been found in the sea and appear to include differences with season and with depth. This may explain the large variations which are to be found in the figures quoted by workers in this field. Both Harvey and Riley have made several estimates of the relevant quantities. Riley (1949, 1952) decides on a factor

$$1\ \text{mg chlorophyll} \equiv 54\ \text{mg carbon}.$$

Harvey's value (1950) is in terms of units of plant pigments which on conversion gives

$$1\ \text{mg chlorophyll} \equiv 27\ \text{mg carbon}.$$

Harvey says of his own figure—'This estimated ratio is no more than a rough approximation. It is unlikely to be less than half or more than double the true mean ratio.'

The ratio given by Riley will be used since one purpose of this paper is to compare certain results obtained here with those of Riley.

Dry Weights of Zooplankton

Since knowledge is required of predation on the plants, some figures must be found that will represent the herbivore population. It is probable that the dry weight of a net haul is the best compromise between simplicity and accuracy.

A Hensen egg-net was used with no. 3 silk (60 meshes per inch). The net was hauled vertically from bottom to surface. The collections were preserved in ethyl alcohol and, on return to the laboratory, dried for 12–18 h at 90° C and then weighed. The values used for the subsequent calculations are the means of the four stations as shown in Fig. 17, with the figure for 21 June found by interpolation.

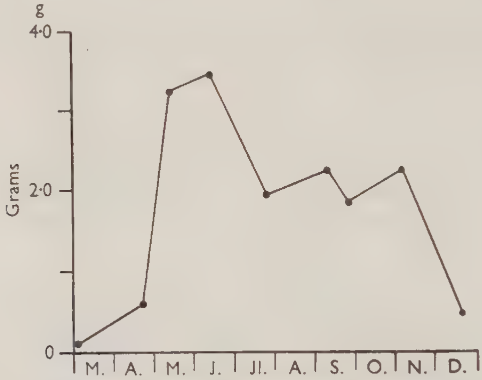

Fig. 17. Mean dry weights of net hauls on Fladen in 1953.

As with chlorophyll data, these results are converted into carbon content. Since the opening of the net has an area of approximately one-third of a square metre, then, assuming 100% filtration and taking the carbon content to be half the dry-weight, the values in Fig. 17 should be multiplied by 1·5 to give carbon content below 1 m^2.

These results are not only very variable, but they are also very much minimum values, since a no. 3 silk catches but a small proportion of the smaller animals. This effect is important when considering the assumptions necessary for using these data in the subsequent calculations.

These data are intended to provide information about the grazing on the plants, and the simplest assumptions which would be required for the calculations are that (*a*) the animals are filter feeders and filter a constant quantity of water per unit of dry weight in a net haul, and (*b*) the zooplankton population spends equal time at all depths.

The most serious objection to (*a*) has been pointed out by Raymont & Gauld (1951) and Gauld & Raymont (1953), who drew attention to the fact that, whereas weight is approximately proportional to the cube of the length, respiration is approximately proportional to the square. Thus the food requirements of the zooplankton will depend on the size frequency-distribution of the population, with the smaller animals having much higher requirements per unit of weight.

Concerning the second assumption, Nicholls (1933) showed that copepodite stages I–III of *Calanus* in Loch Fyne were always most abundant above 30 m during 1932. If this result was regarded as generally applicable, which is not obviously the case, then these two facts together could thus upset the postulates suggested to describe the grazing effects. However, these postulates are not merely the simplest but probably provide the only available approach for linking predation to the plant population.

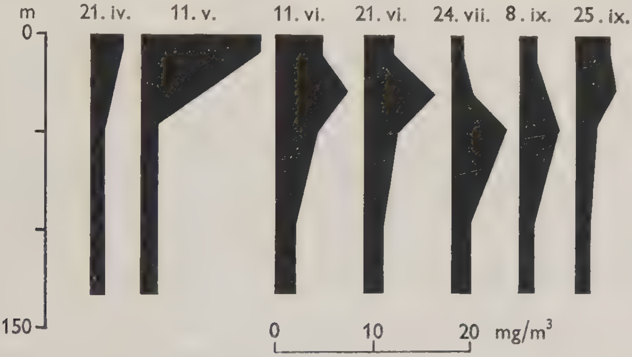

Fig. 18. Profiles of chlorophyll concentration (mg/m³) on Fladen in 1953 constructed from the mean values at 10, 30, 50 and 100 m.

Sinking Rate and Filtering Rate

The value of the constant which converts dry weight to filtering rate will be one of the two unknowns which must first be evaluated. The other initially unknown quantity is the rate at which the plants sink through the water. This and the filtering rate must be evaluated to give a quantitative picture of the reasons for the changing distribution of the plants.

Qualitatively, this sequence of events can be seen in Fig. 18. After the spring outburst, production does not occur at a high rate and, during the summer, the gradual sinking of the population and its grazing down is shown by the successively greater depths at which maximum concentration occurs and by the decreasing total chlorophyll content.

The basic mathematical form which will express these various effects is as follows (see Riley *et al.* 1949):

$$\frac{\partial P}{\partial t}=(p_h-r-gZ)P+\frac{\partial}{\partial z}\left(\frac{A}{\rho}\frac{\partial P}{\partial z}\right)-v\frac{\partial P}{\partial z}, \tag{1}$$

where P = density of the plant population, p_h = photosynthetic rate, r = respiration rate, g = filtering rate, Z = density of herbivore population, A = coefficient of vertical diffusivity, v = sinking rate, z = depth. The units used here are grams, metres, days. The term $(p_h - r)\,P$ can be replaced here by the estimates of production rate derived from the phosphate results.

Riley *et al.* (1949) also made the further assumption throughout their work that $\partial P/\partial t = 0$, which allowed them to study each station separately. It is obvious from the profiles shown in Fig. 18 that the phytoplankton on Fladen cannot be considered as existing in a steady state. Thus the methods used by Riley could not be applied here. It is necessary to consider the changes between two stations and, as with the phosphate, this involves making several approximations. The integrated approximate form of (1) is

$$Pr = \int_{140}^{z} [P_2 - P_1 + \tfrac{1}{2} g \Delta t (Z_1 P_1 + Z_2 P_2)]\, dz - \frac{\partial P/\partial z}{\partial T/\partial z} \int_{140}^{z} (T_2 - T_1)\, dz - \tfrac{1}{2} \Delta t v [P_1 + P_2]_{140}^{z}, \qquad (2)$$

where Pr = production below z metres during the time interval Δt,
P_1, P_2 = population at beginning and end of time interval Δt,
$\partial P/\partial z$, $\partial T/\partial z$ = mean gradients of population and temperature at depth z,
Z_1 and Z_2 = density of herbivore population, and
Z_r is found by dividing the carbon content per square metre by 140.

In (2) *g and v may be regarded as unknown, and the first use which will be made of this equation is to solve for them.* For each interval two equations can be found, corresponding to the depth intervals $0 - z_0$ metres and $z_0 - 140$ m, where z_0 is the zero level. These equations can be solved simultaneously to give values for g and v.

Of the results shown in Table XII, those for the first interval appear to be abnormally high; the reasons for this will be discussed later. Excluding this interval the others show a comparatively small range of variation with mean values of 3·0 m/day and 1·34 m^3/day/g zooplankton carbon.

The sinking rates proposed by Riley were dependent on temperature, but such a variation is small compared with the overall variation of the results and has not been included here. Over the temperature range found here Riley's choice of sinking rate varies between 3·5 and 4·3 m/day. The values given here cover this range but have a lower mean value. However, when one remembers the great range of values for sinking rate which has been found previously (Riley *et al.* 1949, pp. 84–5), the agreement between the figures given here and

TABLE XII. SINKING RATES AND FILTERING RATES

	21. iv.–11. v.	11. v.–11. vi.	11. vi.–21. vi.	21. vi.–24. vii.	24. vii.–8. ix.	8. ix.–25. i
Sinking rate (m/day)	13·0	2·4	2·1	4·2	4·9	1·4
Filtering rate (m^3/day/g carbon)	2·9	1·0	1·2	1·7	1·4	1·4

those of Riley can be taken as very satisfactory. Similarly, the mean filtering rate does not differ greatly from Riley's estimate of 1·26 m^3/day/g C.

With values for filtering and sinking rates available, the detailed shape of the profiles can now be considered. Equation (2) can be put in the form

$$(1+\tfrac{1}{2}g\Delta tZ_2)\int_{140}^{z} P_2\,dz-\tfrac{1}{2}\Delta tvP_2=Pr+(1-\tfrac{1}{2}g\Delta tZ_1)\int_{140}^{z} P_1\,dz$$

$$+\frac{\partial P/\partial z}{\partial T/\partial z}\int_{140}^{z}(T_2-T_1)\,dz+\tfrac{1}{2}\Delta tv\,(P_1-[P_1+P_2]_{140}^{z}), \qquad (3)$$

where

$$\frac{\partial P}{\partial z}=\tfrac{1}{40}[P_1+P_2]_{z+10}^{z-10}. \qquad (4)$$

The left-hand side of (3) contains P_2 alone, but the right-hand side, beside terms in P_1, involves P_2 in the term $\partial P/\partial z$, as shown in (4). The third term on the right-hand side is, however, found to be much smaller than the others and *thus* (3) *can be used to predict* P_2 *by successive approximation.* In fact, because of the low degree of accuracy, no second approximation is necessary.

As a first step, a mean value is found for the 0–20 m layer; then this value is used to find the 30 m value; and so on to the top of the homogeneous bottom layer. In this way a profile is obtained from surface to about 50–70 m. This profile can be compared with the three observed values at 10, 30 and 50 m. With the exception of the first time interval, the computations were first made with the values for sinking and filtering rates given in Table XII. Then, for the first interval and some of the subsequent ones, other values were used in search of a better fit. The results of these calculations are shown in Fig. 19.

It is in the first four cases that the observed points show the greatest changes with depth, so that these cases are of the most interest from the point of view of predicting results.

These four profiles show the same type of distribution with depth as the observed points. The last two profiles also show quite large changes with depth, but these are not borne out by the observed points.

The two profiles which have been drawn for the first interval show that a high filtering rate and low sinking rate are required to give a reasonably shaped profile with approximately the observed quantity of plankton above 50 m. Similarly, increasing the filtering rate in the second and third cases brings the profiles into better agreement with the observed points. In the fourth interval the initial profile is as good a fit as can be obtained. The fifth profile does show an increase with depth which is also true of the observed points; decreasing the sinking rate will give a rather better fit, but because of the small number of observed points there is no confirmation of the predicted maximum at 40 m. In the last case it was not found possible to obtain a reasonable fit.

Returning to the first interval, in both sets of calculations it gives results which are very different from the succeeding intervals. This interval covers the spring outburst and so it is likely that the population will consist largely of juvenile stages of the copepods. This would provide the conditions which, as suggested on p. 27, would not fulfil the postulates on which the grazing effects were based. During this period it is possible that grazing, dominated by the juveniles with their proportionately higher metabolic rate, was confined to the water above 50 m which, in turn, provides an explanation of the anomalous results of the first calculation and of the high filtering rates given by the second. Such a hypothesis might well be necessary since the juveniles, with their proportionally greater food requirements, would perhaps be unable to obtain sufficient food if they spent only a small part of their time in the zone of high plant concentration. Later, when they are adult and when the maximum concentration of phytoplankton may occur at widely varying depths, vertical migration could be expected to be more extensive, and the postulate of equal depth concentration over long time intervals becomes more acceptable.

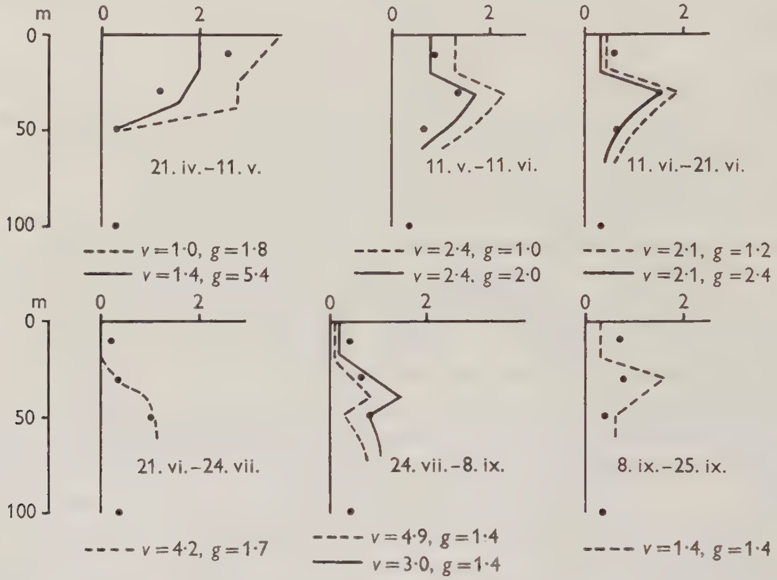

Fig. 19. Comparison of calculated and observed chlorophyll concentrations (mg/m³).

The need for a greater filtering rate is still apparent in May, but by June the differences between observed and calculated values are so small that a greater filtering rate does not provide a significantly better fit. Thus the anomalies between the two calculations decrease from April onwards, perhaps because of the progressive domination of the population by the later stages of copepods.

Regarding the interval 8–25 September, it will be remembered that the phosphate results for this interval were somewhat erratic (p. 16), and it was suggested (p. 16) that there was some shift in the water during this period.

Possibly this could also explain the poor fit for the chlorophyll data on the 25th.

It also seems possible that there are significant changes in the sinking rate. The values deduced here start at about 1·4 m/day, rise to above 4 m/day at the end of July and then in September return to 1·4 m/day. Gross & Zeuthen (1948) have suggested that under suitable conditions diatoms do not sink, since their specific gravity equals that of the surrounding sea water. But to maintain this buoyancy requires the expenditure of energy and so sinking occurs when these energy requirements cannot be met. This situation is most likely to occur in a senescent population. Thus it is possible that the variations in the calculated sinking rate may correspond to the gradual change from a new plant population in April and May, living in a suitable environment, to a senescent population in July, with a return in the autumn to a population of comparatively recent origin.

From this discussion it would appear that neither the sinking rate, v, nor the filtering rate, g, will be constant throughout the year but will depend on the age composition of the phytoplankton and zooplankton respectively.

Finally, it has not proved possible to start with given constant rates of sinking and filtering and from them to predict the cycle of plant distribution throughout the year. Yet it does seem that by varying the sinking and filtering rates from one interval to the next the changes in plant distribution can be largely explained in terms of the factors listed at the outset; further, these variations in the rates of sinking and filtering are not unreasonable.

Conclusions

The aim of the first part of this paper was to develop a method of estimating the basic plant production. The second part was designed to measure the factors which control the distribution of the plants with time and with depth. In each there has been some measure of success, but most of the results are open to question and, further, in this paper the methods have been applied in detail to one area only.

This type of work requires chemical and biological data collected over many years to provide information on the possible kinds of variation that may occur. The results from the northern North Sea cover, at most, three years, with only one year's data for the areas outside Fladen. Thus the problems tackled so far have been concerned with the basic regularities and the more important variations. For this purpose Fladen is very suitable since it appears to give a good approximation to the case of purely vertical mixing.

In the areas outside Fladen the difficulties which arise are due mainly to the effects of lateral water movements. The measurement of these would be required before the changes at, say, the Inshore group could be properly understood. Similarly, features in the Fladen results such as the apparent production between 20 and 30 m in the summer, or the anomalous results in the chlorophyll prediction, probably require knowledge of the metabolism of plants and animals which is not at present available. But these are problems which face any method of studying production.

SUMMARY

In the first part of the paper estimates of the plant production are given for the years 1951–53 in one area, Fladen, of the northern North Sea. These estimates, based on the changes in inorganic phosphate, give values for the yearly production of 55–81 g carbon under each square metre of surface—a range which is nearly the same as that in the English Channel; its variation can be related to the varying occurrence of high winds in the different years. It appears that the zone of phosphate utilization sinks progressively during spring and summer to a depth around 30–40 m.

In the second part these estimates are then used, together with chlorophyll and zooplankton data, (*a*) to study the changes in the vertical distribution of the plants, and (*b*) by comparing calculated and observed vertical chlorophyll profiles, to discover to what extent a few simple postulates can explain the changing vertical distribution of plants throughout one year.

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PORPHYRIN PIGMENTS IN THE TECTIBRANCH MOLLUSC *AKERA BULLATA* O. F. MÜLLER

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The sudden appearance of *Akera bullata* in the static water tanks of H.M. Dockyard, Devonport, provided specimens for the investigation of pigments. Morton & Holme (1955) have given an account of certain aspects of the biology of this interesting tectibranch mollusc.

Fifteen fresh specimens were minced in the Waring blender, and the resulting material was treated in the following ways.

Method 1

A portion of the minced material was extracted with absolute methanol, and the extract filtered. The green solution was intensely red-fluorescent in ultra-violet light, and was submitted to long-paper chromatography (Kennedy, 1953). The solvent phases were 2:6-lutidine (5 parts) and water (3 parts), and the chromatograms were run at 23° C in an atmosphere of ammonia.

When the chromatograms were viewed by ultra-violet light, they revealed a spot, red-fluorescent, with R_F value 0·95, indicating a monocarboxyl porphyrin compound such as phaeophorbide. There was another spot with R_F value 1·0 (i.e. travelling with the solvent), which was red, and had a blue fluorescence. This was most probably carotenoid with fat.

The main purpose of this preliminary experiment was to determine the presence or absence of unchanged chlorophyll in the animal. The fact that no red-fluorescent spot with an R_F value 1·0 was found, indicated the absence of this pigment.

Method 2

A portion was extracted with a mixture of methanol and concentrated sulphuric acid (19:1) and allowed to stand overnight in the ice-chest. It was then diluted with an equal volume of water, and the pigmented material was extracted with Analar chloroform, giving an extract which was intensely fluorescent when viewed in ultra-violet light. This extract was roughly dried by passing it through chloroform-soaked paper, and evaporated to dryness *in vacuo*. The residue was redissolved in dried Analar chloroform, and chromatographed on magnesium oxide grade III (Nicholas, 1951). A deep red, red-fluorescent, band appeared on development with chloroform which passed slowly down the column and was collected as Fraction A (Kennedy, 1953). A deep green, non-fluorescent, band remained on the column and could not be eluted with graded chloroform/methanol mixtures.

Fraction A was evaporated to dryness *in vacuo*. A portion of the residue was dissolved in Analar pyridine, and the remainder in Analar chloroform. Spectrophotometric examination of the pyridine solution gave the following absorption maxima:

I	II	III	IV	Soret band
626	572	536	502	405·5 mμ

A specimen of uroporphyrin I, in pyridine, gave the following maxima:

627	571	536	501	406 mμ

Fraction A was, therefore, considered to be uroporphyrin. Examination of this fraction by the ascending paper chromatography methods of Chu, Green & Chu (1951) and of Falk & Benson (1953) showed that the uroporphyrin was present as isomer I.

The deep-green non-fluorescent material remaining on the column was eluted with ethyl acetate, which was later removed *in vacuo*. The residue was re-dissolved in dry chlorofrom, and chromatographed on alumina (B.D.H. chromatographic grade). Two bands were obtained, the top one green, and the lower one yellow. The green band was entirely non-fluorescent. The yellow band was slightly yellow-fluorescent, suggesting carotenoids, and gradually passed through the column to give a solution with an indeterminate spectrum.

On further development with chloroform the whole column became green-blue and the washings were colourless. After many attempts the green-blue pigment was finally eluted with a mixture of *n*-butanol and formic acid (50:0·5). The formic acid was removed from the eluate with water, using the smallest volumes possible, and adding compensatory volumes of *n*-butanol to avoid losing pigment. The butanol was then removed *in vacuo*. The pigment residue could then be dissolved in chloroform, and was flocculated with light petroleum (b.p. 40–60° C), giving a dark-green product. This was dried *in vacuo*, and a portion redissolved in chloroform gave the following maxima (in the Hartridge reversion spectroscope):

I	I	III	IV
655·3	604·0	554·2	465·5 downwards mμ

A sample dissolved in toluene and examined in the Unicam spectrophotometer gave the following maxima:

660	559	427 mμ

This pigment gave none of the typical bile pigment reactions. The absorption curve, together with the absence of fluorescence, suggested that the pigment might be a metal complex of a chlorophyll derivative.

Metals may frequently be removed from metalloporphyrins by treatment with acids or with hydrazine, and therefore the green pigment was warmed with concentrated hydrochloric acid on a water-bath, and driven into ether by adding saturated potassium acetate. The pigment was unchanged, which suggests the probable absence of zinc, divalent tin and silver. Treatment with concentrated sulphuric acid produced some red fluorescence which rapidly disappeared, due undoubtedly to the destruction of the pigment. Concentrated sulphuric acid is known to remove copper, divalent iron, nickel and cobalt. It is therefore possible that one of these metals could be present.

On dissolving the green pigment in propionic acid and boiling with hydrazine for several minutes, the mixture gradually acquired a fine red fluorescence (when viewed with ultra-violet light) and gave an absorption spectrum (Hartridge reversion spectroscope) consisting of one very strong band at 662 mμ and two weak bands at 540 and 509 mμ respectively. This indicates that the pigment could be a metal derivative of phaeophorbide *a*.

A chemical test for metals was kindly carried out by Mr F. A. J. Armstrong. The pigment gave 30% residue after ignition, and a very strong reaction for copper. There was a trace of iron, but no nickel.

A sample of phaeophorbide *a*, prepared from chlorophyll *a*, was dissolved in boiling ethanol, and while boiling, freshly precipitated copper powder was added, the whole operation being conducted under ultra-violet light. The intense red-fluorescence of the phaeophorbide disappeared almost at once, and, after boiling for a further 60 sec, and examination in chloroform in the Hartridge Reversion Spectroscope, the spectrum was found to be:

I	II	III	IV	
656·0	604·0	554·0	465	mμ

This agrees well with the spectrum of the non-fluorescent pigment in chloroform.

A sample of the ethanolic solution was evaporated to dryness and dissolved in toluene, and the spectrum examined in the Hartridge Spectroscope:

661 560 427 mμ

Thus it is reasonable to deduce that the two pigments—natural and synthetic—are identical, that is, the non-fluorescent green pigment of *Akera* is a copper co-ordination complex of phaeophorbide *a*.

Method 3

The second portion of the original fresh minced material was extracted with a mixture of ether and acetic acid (5:1), and gave a green solution which was intensely red fluorescent. The acetic acid was washed out with water containing 0·05% of Teepol detergent; this was used to avoid emulsification (A. S. C. Lawrence, personal communication, 1953). The remaining ether solution was extracted successively with 1, 8 and 15%, w/v, hydrochloric acid. The 1 and 8% acids extracted no fluorescent material, thus indicating the absence of coproporphyrin and protoporphyrin. The 15% hydrochloric acid extract was, however, deep green-blue, and intensely red-fluorescent, suggesting the presence of free phaeophorbides, which have HCl numbers of 15 (phaeophorbide *a*) and 19·5 (phaeophorbide *b*) (Willstätter & Stoll, 1913). The whole ether solution, therefore, was completely extracted with 15% hydrochloric acid (uroporphyrin is not extracted by the ether/acetic acid method, and therefore remains in the original minced material).

The 15% hydrochloric acid extract was repeatedly shaken with chloroform, and the deep-blue hypophases pooled. The chloroform solution was washed with water and roughly dried by passing through chloroform-soaked paper, and evaporated to dryness *in vacuo*. An iridescent blue-black residue was obtained, which was re-dissolved in dry peroxide-free ether and fractionated with 0·2% aqueous sodium bicarbonate. Phaeophorbide *b*, being slightly more acidic than phaeophorbide *a*, is extracted quantitatively from a mixture of the two by 0·2% aqueous sodium bicarbonate (Willstätter & Stoll, 1913). No fluorescent hypophases, however, were obtained, indicating the presence of phaeophorbide *a* only. The ether solution was evaporated *in vacuo* and the residue redissolved in Analar chloroform. Examined in the Hartridge reversion spectroscope this solution gave the following absorption maxima:

I	II	III	IV	
666·6	606·7	539·2	507·3	mμ

This is clearly phaeophorbide *a*.

DISCUSSION

Kennedy & Vevers (1954) found uroporphyrin I in the integument of *Aplysia punctata*, and it is noteworthy, though not surprising, that the same porphyrin was isolated from *Akera*, a related form.

It is curious that only phaeophorbide *a* was detected, in view of the fact that *Akera*—like *Aplysia*—grazes on *Ulva* (Morton & Holme, 1955) which contains chlorophyll *a* and *b*. Dales & Kennedy (1954) reported a similar result from *Nereis diversicolor*. Both chlorophylls *a* and *b* must be ingested by the animals, and it is very difficult to account for the absence of the *b* degradation component. The reason may lie, as far as *Akera* is concerned, in the fact that phaeophorbide *b* is difficultly soluble in ether, and, if present originally in very small amounts, as is most likely (the ratio of chlorophyll *a* to chlorophyll *b* is often as high as 3:1), the pigment may have been washed out in the process of removal of the acetic acid. This explanation is not really satisfactory, and in some further work the full reason will be sought.

The presence of a copper complex of phaeophorbide *a* is of great interest. Some molluscs are known to concentrate copper from the sea, and of course the respiratory pigment haemocyanin, which is a copper-polypeptide, occurs in the cephalopod and gastropod molluscs, and in the arthropods. However, there is no doubt that the green non-fluorescent pigment isolated from *Akera*, is a copper phaeophorbide. That this is not an artifact, caused by the co-ordination of copper ions with phaeophorbide in the course of extraction, is supported by the fact that free phaeophorbide also occurs, whereas the affinity of copper for porphyrin pigments is so great that if free copper were present in the animal, *all* the phaeophorbide would have become changed to the metal complex. The reagents used were, of course, beyond reproach, but the same thing would have been true had they contained copper salts or impurities—*all* the phaeophorbide would have formed the copper derivative. In some further work on the pigments of *Aplysia*, to be completed in the near future, J. E. Morton and G. Y. Kennedy will show that a similar pigment occurs in that animal.

SUMMARY

Some pigments of *Akera bullata* (O. F. Müller) are described with methods for their isolation. The pigment of the integument is uroporphyrin I, and from the viscera phaeophorbide *a* together with its non-fluorescent copper complex were isolated. Some points of interest concerning these pigments are discussed.

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VITAMIN A AND CAROTENOIDS IN CERTAIN INVERTEBRATES

IV. MOLLUSCA: LORICATA, LAMELLIBRANCHIATA, AND GASTROPODA

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INTRODUCTION

Apart from a general account (Kon, 1954) of our work on vitamin A and carotenoids in invertebrates, our publications so far (Kon & Thompson, 1949*a*, *b*; Batham, Fisher, Henry, Kon & Thompson, 1951; Fisher, Kon & Thompson, 1951, 1952, 1953, 1954, 1955) have been concerned only with marine Crustacea. We have also studied, during our investigation of the metabolism of vitamin A and its possible precursors in the sea, numerous species from most other phyla of marine invertebrates. Except for the nematode worm, *Anisakis physeteris* Baylis, taken from the stomach of a sperm whale, the only two phyla of invertebrate animals in which we have so far found vitamin A are the Arthropoda and the Mollusca. The vitamin was present in at least some species from each of the molluscan classes, Loricata, Gastropoda, Lamellibranchiata and Cephalopoda, but we have no information yet about the Solenogastres or the Scaphopoda of which we have analysed no representatives. So far as our studies were concerned, the cephalopods differed considerably from the other molluscs examined, and the relatively large amount of information they have provided will be more conveniently presented in a subsequent paper. The account which follows, therefore, deals only with species from the first three classes just listed.

To the best of our knowledge, Jameson, Drummond & Coward (1922) were the first to report vitamin A activity in molluscs, but they did not name the species they had tested. Since that date all the published work on vitamin A known to us has been concerned with the food value of clams (Jones, Nelson, Murphy & Devine, 1928) and oysters, which were analysed in the United States by Jones & Murphy (1926), Jones, Murphy & Nelson (1928) and Whipple (1935), in New Zealand by Malcolm (1926, 1927, 1928) and in Malaya by Leong (1939). All these reports gave values for vitamin A activity rather than for vitamin A itself, since they were based on biological tests of whole specimens without previous separation of the vitamin from its precursors. The presence of vitamin A in Loricata and in Gastropoda has not previously been reported.

The history of the investigation of molluscan carotenoids began with the work of Merejkowsky (1881, 1883) and MacMunn (1883*a*, *b*) who extracted a red fat-soluble pigment they called tetronerythrin from many marine invertebrates, including several species of molluscs. Tetronerythrin had the properties of carotenoids, and probably represented the sum total of these pigments in extracts. No further work on the carotenoids of molluscs was reported until the appearance of Lönnberg's papers (Lönnberg, 1931, 1934*a*, *b*; Lönnberg & Hellström, 1932), in which were given the absorption spectra of pigments extracted from many species of marine invertebrates including several molluscs. More specialized studies of carotenoid metabolism in molluscs have been made by Scheer (1940) on mussels, Brooks & Paulais (1939) on oysters, and Goodwin (1950) and Goodwin & Taha (1950, 1951) on limpets. Goodwin (1953) has also investigated the carotenoids of the freshwater mussel, *Anodonta cygnea*.

Our own work has been concerned primarily with the detection and measurement of vitamin A in molluscs, and carotenoids have only been identified and measured when they were separated in our normal technique of analysis.

MATERIAL COLLECTED

Eighteen species of Gastropoda, nine species of Lamellibranchiata and one species of Loricata were collected, many of them by ourselves when visiting the shores of different parts of Great Britain, and during a visit by one of us (S. K. K.) to the Scripps Institution of Oceanography, at La Jolla, California, in May 1953. The staff of various marine biological and fisheries laboratories have kindly collected much material for us or allowed us facilities to do our own collecting.

From La Jolla we had the following species: *Cypraea spadicea* Swainson, *Haliotis fulgens* Philippi, *Megathura crenulata* (Sowerby), *Astrea undosa* (Wood) and *Stenoplax conspicua* (Carpenter). We obtained *Lima hians* (Gmelin) and *Pecten maximus* (Linnaeus) at Millport, *Mya arenaria* Linnaeus and *Cardium edule* Linnaeus from Fairlie Sands, and *Aporrhais pes-pelecani* (Linnaeus) and *Chlamys septemradiatus* Müller from Loch Fyne. More species were collected on various parts of the Essex coast; these included *Scrobicularia plana* (da Costa) taken from mud flats at Leigh-on-Sea, *Ostrea edulis* Linnaeus, *Gryphea angulata* Lamarck and *Crepidula fornicata* (Linnaeus) from the rivers Crouch and Roach near Burnham, and *Littorina littoralis* (Linnaeus), *L. littorea* (Linnaeus), *L. rudis* (Maton), *Patella vulgata* Linnaeus and *Mytilus edulis* Linnaeus from the breakwater at Blackman's Point, Harwich. Groups of *Buccinum undatum* Linnaeus were collected at both Millport and Burnham-on-Crouch. *Osilinus lineatus* (da Costa) was collected for us at Plymouth. *Aplysia depilans* Linnaeus and *Murex trunculus* Linnaeus were obtained from collections of marine animals received by the London Zoological Society's aquarium from Madeira. The specimens of

Clione limacina Phipps were taken during a cruise of F.R.S. *Scotia* to Iceland in August 1951, and those of *Limacina retroversa* (Fleming) from R.V. *Ernest Holt* in the Barents Sea in January 1955. *Helix aspersa* Linnaeus was collected from a garden in Shinfield and *Planorbis corneus* (Linnaeus) var. *rubra* Oldham was purchased from an aquarist in Reading. These pulmonates, although not marine, were included for comparative purposes. The red variety of *P. corneus* seemed to be especially interesting in view of its apparent carotenoid colouring.

METHODS OF PRESERVATION

Apart from the two planktonic species, *Clione limacina* and *Limacina retroversa*, which were preserved in the same way as Crustacea, by immersion in boiling sea-water for 2 min. (Fisher *et al.*, 1952), all the material was preserved either whole, or various organs separately, in absolute alcohol. On receipt at the laboratory the boiled specimens were weighed, preserved in alcohol and stored in the deep-freeze. Where possible, specimens or organs preserved in alcohol were weighed first. If not, a known volume of absolute alcohol was added so that the weight of the animals could be determined by difference, that of the alcohol being calculated from its specific gravity. Where specimens were sent to us in an unknown volume of alcohol the net weight was roughly determined by draining off the alcohol and weighing the specimens. Weights given in the results and tables were determined after removal of the shells, except for *Stenoplax conspicua* and *Limacina retroversa.*

ANALYTICAL METHODS

Basically the analytical technique was that outlined by Fisher *et al.* (1952). Some difficulty was experienced in the Carr-Price reaction owing to interference by xanthophylls which were eluted with vitamin A alcohol in the chromatography following saponification. Recently we have found that some of this pigment may be removed at an early stage by passing the extract in *n*-hexane solution through an alumina column weakened with 8% ethanol in *n*-hexane before the chromatography on a column not so treated as used in method (*b*) of Fisher *et al.* (1952). A large proportion of the other carotenoid pigments is eluted with the vitamin A ester and β-carotene, leaving vitamin A alcohol and a reduced amount of pigments on the column. These are eluted with 8% ethanol in *n*-hexane. The first fraction is then re-chromatographed on an untreated alumina column. Vitamin A ester and β-carotene are eluted by 2% acetone in *n*-hexane, leaving the remaining pigments adsorbed on to the column.

So far in our work vitamin A has been measured by the blue colour produced in the antimony-trichloride reaction and the values determined as described by Thompson (1949). Since pure crystalline vitamin A is now readily available the photoelectric spectrophotometer has been recalibrated with solutions in chloroform of the alcohol, acetate and palmitate forms of vitamin A.

We found that these pure preparations gave 20% less blue colour per unit of vitamin A than the oils originally used as standards. The probable presence in these original oils of about 25% of neo-vitamin A (Dalvi & Morton, 1951) may account in part, at least, for the difference. Pure vitamin A alcohol now gives, under our conditions, a value for $E^{1\%}_{1\,\mathrm{cm.}}$ (620 mμ) of 5000, in sensible agreement with that of 5070 given by Cama, Collins & Morton (1951).

Since the method of measuring vitamin A is purely chemical it seems logical to express results in terms of micrograms rather than international units. This will be done in the present and future papers of this series except where a measurement is the result of a biological assay, when we shall continue to use international units.

To compensate for the recalibration of the spectrophotometer and to convert international units to micrograms the factor 0·39 should be used to multiply all values resulting from chemical measurements of vitamin A given in our previous papers (Thompson, Ganguly & Kon, 1949; Kon & Thompson, 1949*a*, *b*; Batham *et al.*, 1951; Kon, 1954; Fisher *et al.* 1951, 1952, 1953, 1954, 1955).

Our earlier work (Fisher *et al.*, 1952) on euphausiids showed a discrepancy between the vitamin A potencies measured by physico-chemical and by biological methods. We now know that this discrepancy is due to the presence of a vitamin A isomer (Kon, 1954). No biological tests have been done on vitamin A from the molluscs analysed, so that no information is available regarding the nature of the vitamin in them.

RESULTS

Class Loricata

Stenoplax conspicua (Carpenter)

Ten specimens were collected on the shore at La Jolla on 20 May 1953. The shells were not removed before analysis and the results were as follows:

Average weight (g)	Oil (%)	Vitamin A		Total carotenoids		β-carotene
		μg/spec.	μg/g	μg/spec.	μg/g	μg/g
17	1·2	0·73	0·044	85	5·1	0·41

The vitamin A was all in the ester form. Their chromatographic behaviour indicated that the carotenoid pigments included both carotenes and xanthophylls and possibly astaxanthin or its esters. Chlorophyll was also present.

Class Lamellibranchiata

Order Filibranchiata

Mytilus edulis Linnaeus

Three groups of mussels were collected near the breakwater at Blackman's Point, Harwich. The analytical results are shown in Table 1. In the whole animals only vitamin A ester was measured, since the presence of much pigment in the fraction that would have contained vitamin A alcohol prevented a satisfactory reading in the Carr-Price reaction in the first two groups of specimens.

The pigmented edge of the mantle is believed to be light-sensitive in some molluscs and, because of our finding that vitamin A is concentrated in the eyes of Crustacea (Fisher *et al.*, 1951), this mantle edge was separated from the rest of the mussel before analysis of the second group. It contained no vitamin A, and the concentration of fat and total carotenoids differed little from those of the rest of the mussel, which, however, contained vitamin A ester.

TABLE I. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *MYTILUS EDULIS*

(All specimens collected at Harwich.)

Date	Tissue	No.	Average weight (g)	Oil (%)	Vitamin A		Total carotenoids		β-carotene (μg/g)
					μg/spec.	μg/g	μg/spec.	μg/g	
11. ix. 50	Whole animal	29	1·3	1·6	0·66	0·51	49	37	1·3
29. i. 51	Mantle edge	40	0·1	1·1	0	0	4	34	0
	Visceral mass	40	1·4	1·5	0·25	0·18	43	32	0·77
	Total	40	1·5	1·5	0·25	0·16	47	32	0·70
23. iv. 51	Digestive gland	36	0·45	1·2	0·58	1·29	91	203	14
	Mantle, with eggs	36	0·55	1·1	0·04	0·08	16	29	0
	Foot	36	0·04	3·2	0	0	2·6	74	0
	Ctenidia, muscle and rest of visceral mass	36	0·77	0·9	0·04	0·05	27	34	1·1
	Total	36	1·81	1·1	0·66	0·37	137	76	4·2

The mussels of the third group were dissected into several parts before analysis and, as Table I shows, vitamin A was concentrated mainly in the digestive gland where it was in the alcohol form. The small amounts of vitamin A in other parts of the animal were present entirely as the ester. Apart from β-carotene, the carotenoids were xanthophylls, with no evidence of astaxanthin. There was a trace of chlorophyll in the digestive gland.

Pecten maximus (Linnaeus)

The analytical results for various parts of a group of seven specimens are shown in Table II. The carotenoids were mainly xanthophylls and no astaxanthin was detected.

Chlamys septemradiatus Müller

Table II shows results for a group of whole animals of this species. The only carotenoids present were xanthophylls, and considerable amounts of chlorophyll were also observed.

Lima hians (Gmelin)

Two groups were analysed, the first as whole animals and the second after dissection. The results are shown in Table III. Vitamin A was present in the first group, all in the ester form, but not in the second. The carotenoids were all xanthophyllic, apart from traces of a carotene in the digestive gland.

TABLE II. DISTRIBUTION OF OIL AND CAROTENOIDS IN TWO SPECIES OF PECTINIDAE

(Both species collected in Clyde Sea area.)

Date	Species and tissue	No. of specimens	Average weight (g)	Oil (%)	Total carotenoids		β-carotene (µg/g)
					µg/spec.	µg/g	
13. ii. 51	*Pecten maximus*	7	—	—	—	—	—
	Muscle		56	0·3	28	0·5	Trace
	Mantle edge		19	0·3	9·2	0·5	Trace
	Visceral mass		9·5	4·2	364	39	0·58
	Gonad and foot		13	1·9	186	14	Trace
	Ctenidia and mantle		7·4	0·5	62	8·4	0
	Total		105	0·9	649	6·2	0·05
13. iii. 52	*Chlamys septemradiatus*	31	1·5	0·6	3·6	2·4	0

Vitamin A absent from both species.

TABLE III. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *LIMA HIANS*

(All specimens collected near Millport.)

Date	Tissue	No.	Average weight (g)	Oil (%)	Vitamin A		Carotenoids	
					µg/spec.	µg/g	µg/spec.	µg/g
21. x. 53	Whole animal	12	7·5	1·7	0·20	0·022	188	25
7. xii. 53	Foot	27	0·1	0·5	0	0	2·8	29
	Digestive gland	27	0·7	6·1	0	0	31	44
	Visceral mass	27	1·7	1·1	0	0	5·8	3·4
	Mantle	27	3·6	0·6	0	0	64	18
	Total	27	6·1	1·4	0	0	104	17

β-carotene absent from all specimens.

Ostrea edulis Linnaeus

The analytical results for three groups of edible oysters are given in Table IV. Only vitamin A ester was measured in the whole animals, owing to interference of carotenoids with the measurement of any vitamin A alcohol that might have been present. In the dissected groups satisfactory readings were obtained for all organs. The visceral masses contained vitamin A ester but no alcohol, and the carotenoids were mainly xanthophylls with small amounts of β-carotene. The carotenoids of the remainder of the body were all xanthophylls. An unidentified olive-green pigment was strongly adsorbed at the top of the alumina column in the extracts of whole oysters and visceral masses.

Table IV. Distribution of Oil, Vitamin A and Carotenoids in *Ostrea edulis*

(All specimens collected near Burnham-on-Crouch.)

Date	Tissue	No.	Average weight (g)	Oil (%)	Vitamin A		Total carotenoids		β-carotene (µg/g)
					µg/spec.	µg/g	µg/spec.	µg/g	
10. x. 50	Whole animal	4	9·2	2·3	2·4	0·26	181	20	1·7
11. xii. 50	Visceral mass	16	2·6	1·2	0·35	0·14	12	46	0·05
	Mantle edge	16	0·8	0·5	0	0	1·0	1·2	0
	Muscle	16	1·8	0·5	0	0	1·2	0·7	0
	Ctenidia	16	1·3	0·2	0	0	0·8	0·6	0
	Total	16	6·5	0·7	0·35	0·05	15	2·3	0·02
25. iii. 52	Digestive gland	16	0·5	1·7	0	0	4·8	9·8	0
	Mantle & ctenidia	16	2·5	0·7	0	0	2·5	1·2	0
	Visceral mass	16	2·2	1·8	0·43	0·20	27	12	0·02
	Muscle	16	1·1	0·5	0	0	0	0	0
	Total	16	6·3	1·1	0·43	0·07	34	5·4	0·007

Table V. Distribution of Oil, Vitamin A and Carotenoids in *Gryphaea angulata*

(All specimens collected near Burnham-on-Crouch.)

Date	Tissue	No.	Average weight (g)	Oil (%)	Vitamin A		Total carotenoids		β-carotene (µg/g)
					µg/spec.	µg/g	µg/spec.	µg/g	
10. x. 50	Whole animal	2	12	2·4	1·9	0·16	180	15	1·1
11. xii. 50	Visceral mass	7	15	2·3	5·4	0·35	270	18	0·36
	Mantle edge	7	2·0	0·9	0	0	1·8	0·9	0
	Muscle	7	5·1	0·5	0	0	2·0	0·4	0
	Ctenidia & mantle	7	7·2	1·1	0	0	6·2	0·9	0
	Total	7	29	1·6	5·4	0·18	280	9·4	0·19
27. ii. 51	Labial palps	30	0·7	3·6	0·20	0·30	1·3	1·9	Trace
	Digestive gland	30	1·7	2·2	1·4	0·83	229	134	3·1
	Visceral mass	30	4·5	2·0	0·64	0·14	16	3·5	0·07
	Muscle, mantle & ctenidia	30	9·6	0·7	0·29	0·03	4·3	0·5	0
	Total	30	17	1·4	2·6	0·15	251	15	0·34
25. iii. 52	Mantle & ctenidia	15	2·5	1·0	0·14	0·06	4·4	1·8	0
	Visceral mass	15	3·2	2·0	2·1	0·66	68	21	0·11
	Muscle	15	1·0	0·4	0	0	0	0	0
	Digestive gland	15	0·78	2·6	0·82	1·1	39	51	0·49
	Total	15	7·5	1·5	3·1	0·41	111	15	0·06

Gryphaea angulata Lamarck

Table V shows the analytical results for four groups of Portuguese oysters. In the analysis of the whole specimens the chromatographic behaviour of the pigments was very similar to that in *Ostrea* and the concentrations of fat, vitamin A, total carotenoids and β-carotene were of the same order in both kinds of oyster. The green pigment found in *Ostrea* was also present in *Gryphaea*.

The results for the second group were very similar to those obtained for the group of *Ostrea edulis* collected at the same time and dissected in the same way, except that the vitamin A of the visceral mass comprised about 6 parts alcohol to 1 part ester, whereas in *Ostrea* it was all in the ester form. Analysis of various organs in the third group showed that this species differed from *Ostrea* in having most of the vitamin A in the digestive gland, where the ester-alcohol ratio was 1:5. In the rest of the visceral mass the ratio was 2:1, and in other parts of the body containing it the vitamin was entirely in the ester form. The relative amounts of the two forms of vitamin A in the various parts of the last group were similar to those in the first three, except that in the visceral mass there was over twice as much vitamin A alcohol as ester. Xanthophylls and carotenes were present in all the organs from all the groups, but no astaxanthin.

TABLE VI. DISTRIBUTION OF OIL AND CAROTENOIDS IN *SCROBICULARIA PLANA*

(All specimens collected at Leigh-on-Sea.)

Date	Tissue	No.	Average weight (g)	Oil (%)	Total carotenoids		β-carotene (μg/g)
					μg/spec.	μg/g	
16. x. 50	Whole animals	46	1·55	0·9	38	22	1·0
29. i. 51	Visceral mass	32	1·22	1·3	14	12	0·42
	Siphon	32	0·37	1·4	3·5	9·6	0
	Mantle edge	32	0·23	1·1	0·4	1·6	0
	Total	32	1·82	1·3	18	9·9	0·28

Vitamin A absent.

Order Eulamellibranchiata

Scrobicularia plana (da Costa)

As shown in Table VI two groups of this species were analysed. Carotenoids were mainly xanthophyllic with some carotenes in the visceral mass. The green pigment noticed in the oysters also appeared in this species.

Cardium edule Linnaeus

Of five groups of cockles analysed, only the first, as Table VII shows, contained any vitamin A. As in the previous species, the carotenoids were mostly xanthophylls. The gonads, separated in the second group, contained a large proportion of the total carotenoids.

Mya arenaria Linnaeus

Four groups of clams were analysed and the results are given in Table VIII. In the first group, analysed whole, only vitamin A ester was measured, because of interference of carotenoids in the determination of the alcohol fraction, but in the subsequent groups vitamin A alcohol was absent from all the organs.

TABLE VII. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *CARDIUM EDULE*

(All specimens collected from Fairlie Sands.)

			Average weight	Oil	Vitamin A		Total carotenoids		
Date	Tissue	No.	(g)	(%)	μg/spec.	μg/g	μg/spec.	μg/g	β-carotene (μg/g)
16. v. 51	Whole animal	20	4·0	0·8	0·66	0·16	47	12	0·34
4. ix. 51	Foot	22	0·25	0·8	0	0	1·8	7·5	0
	Gonad	22	1·86	1·2	0	0	15	7·9	0
	Mantle, muscles and ctenidia	22	1·71	0·2	0	0	3·0	1·8	0
	Visceral mass	22	0·36	0·5	0	0	4·7	13	0·38
	Total	22	4·2	0·7	0	0	24	5·8	0·03
16. x. 51	Mantle, muscles and ctenidia	30	2·12	0·8	0	0	6·9	3·3	0
	Digestive gland	30	0·40	1·1	0	0	9·4	23	0·70
	Visceral mass and foot	30	2·49	0·5	0	0	8·4	3·4	0
	Total	30	5·0	0·7	0	0	25	4·9	0·06
24. iv. 52	Crystalline style	25	0·02	0·5	0	0	0	0	0
	Digestive gland	25	0·48	0·9	0	0	5·4	11	0·22
	Visceral mass and foot	25	2·22	0·4	0	0	4·6	2·1	0
	Mantle, muscles, ctenidia, siphons	25	1·36	0·8	0	0	4·1	3·0	0
	Total	25	4·1	0·6	0	0	14	3·5	0·03
24. iv. 52	Whole animal	25	4·8	0·4	0	0	14	2·9	0·04

TABLE VIII. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *MYA ARENARIA*

(All specimens collected from Fairlie Sands.)

			Average weight	Oil	Vitamin A		Total carotenoids		
Date	Tissue	No.	(g)	(%)	μg/spec.	μg/g	μg/spec.	μg/g	β-carotene (μg/g)
16. v. 51	Whole animal	6	34	0·7	0·80	0·02	527	16	0·39
4. ix. 51	Visceral mass	12	5·9	1·5	0·81	0·14	36	6·0	0·12
	Siphons	12	14	0·4	0·18	0·01	27	1·9	0·03
	Mantle, muscle and ctenidia	12	14	0·6	0·35	0·03	88	6·4	0·25
	Digestive gland	12	1·7	1·7	0·15	0·09	130	75	9·5
	Foot	12	0·4	0·8	0	0	2·3	5·8	0
	Total	12	36	0·5	1·5	0·04	283	7·8	0·58
16. x. 51	Visceral mass and foot	9	2·5	1·4	0·74	0·11	86	13	0·18
	Siphons	9	14	0·2	0·16	0·01	25	1·8	Trace
	Mantle, muscle and ctenidia	9	11	0·4	0·19	0·02	26	2·3	0
	Digestive gland	9	6·8	2·0	0	0	161	64	2·5
	Total	9	34	0·7	1·1	0·03	298	8·8	0·22
12. ii. 52	Crystalline style	18	0·18	0·01	0	0	0	0	0
	Visceral mass	18	5·9	1·2	0·34	0·06	13	2·2	Trace
	Mantle, muscle, ctenidia, siphons	18	18	0·4	0·13	0·007	22	1·2	Trace
	Digestive gland	18	1·3	1·2	0	0	1·8	1·4	0
	Foot	18	0·33	1·0	0	0	1·1	3·2	0
	Total	18	26	0·6	0·47	0·02	38	1·5	Trace

Vitamin A was present in the digestive gland of the second group, but absent from it in the last two groups. The carotenoids were much as in other lamellibranchs, with xanthophylls predominating. Variations in concentration between different organs are shown in the table.

Class Gastropoda

Subclass Prosobranchiata

Order Diotocardia

Haliotis fulgens Philippi

Two specimens were analysed singly, with the results given in Table IX. No vitamin A ester was detected. The presence of much xanthophyllic pigment in the alcohol fractions interfered with the measurement of vitamin A alcohol. The fractions were therefore passed through a column of calcium carbonate, using light petroleum (b.p. 40–60° C) as a solvent and eluent in an attempt to remove the pigment. Some of it, however, was still eluted and the fractions were dissolved in *n*-hexane and their absorption spectra determined in the Beckman spectrophotometer. Both fractions showed maxima at about 327 mμ, indicating the presence of vitamin A alcohol. The values for vitamin A were calculated after appropriate corrections for irrelevant absorption by the procedure of Cama *et al.* (1951). The pigments were carotenes and xanthophylls, astaxanthin being absent. A noticeable feature was the high proportion (*ca.* 20%) of β-carotene in the total carotenoids.

TABLE IX. OIL, VITAMIN A AND CAROTENOIDS IN SOME PROSOBRANCHS

(First three species collected at La Jolla on 20. v. 53 and *Osilinus lineatus* at Plymouth on 9. vi. 52.)

Species	No.	Average weight (g)	Oil (%)	Vitamin A µg/spec.	Vitamin A µg/g	Total carotenoids µg/spec.	Total carotenoids µg/g	β-carotene (µg/g)
Haliotis fulgens	1	268	0·6	11	0·04	1066	4·0	0·84
H. fulgens	1	122	0·7	4·6	0·04	1034	8·5	1·7
Megathura crenulata	1	179	0·4	9·2	0·05	1863	10	3·0
Astrea undosa	1	8·7	1·0	0	0	26	3·0	Trace
Osilinus lineatus	50	1·1	1·1	0·12	0·10	58	52	6·1

Megathura crenulata (Sowerby)

One specimen was analysed with the results shown in Table IX. Vitamin A was all in the alcohol form which was measured spectrophotometrically, because of the interference by carotenoids with the antimony-trichloride reaction. The pigments were similar to those of *Haliotis*, but the percentage (30) of β-carotene was even higher in this species.

Astrea undosa (Wood)

The analytical results for a single specimen are given in Table IX. The pigments were mainly xanthophyllic.

Osilinus lineatus (da Costa)

A group of fifty whole specimens was analysed with the results shown in Table IX. Vitamin A was in the ester form and the carotenoids were xanthophylls and carotenes.

Patella vulgata Linnaeus

The analytical results for all the limpets examined are shown in Table X. Analysis of the first group established the presence of vitamin A, although only the ester was measured. Nearly a third of the total carotenoids in this group was β-carotene.

The second group was analysed with mantle edge and tentacles, as possible photoreceptors, separated from the rest of the animals, but all the vitamin was in the main part of the body.

The tentacles and mantle edge had lower concentrations of carotenoids than the rest of the animal and β-carotene was absent from the tentacles.

The distribution of vitamin A and carotenoids in the limpet was further investigated in the third group. These animals were dissected as shown in Table X. The highest concentration and largest amount of vitamin A were in the digestive gland and excretory organ and the ratio of ester to alcohol was 1:9. In the intestine the ratio was about 1:5.

Vitamin A in the gonads was all in the ester form, and the content and concentration in the testis were two or three times higher than in the ovary. The carotenoid concentration was also much higher in the testis than in the ovary despite the more darkly pigmented appearance of the latter. More than half of the ovarian carotenoids consisted of β-carotene, but in the testis this pigment accounted for little more than a quarter of the total.

By far the highest concentrations of carotenoids were in the digestive gland, excretory organ and intestine. Xanthophylls and carotenes were present in all the extracts.

The ratios of vitamin A alcohol to ester and the proportions of different carotenoids were very similar in the group collected on 24 March 1952 to those of the preceding group. Before the two groups obtained on 12 May 1952 were analysed they were kept alive for 24 hr. and the faeces were collected. Scrapings were also taken of the algae encrusting the rocks on which the limpets were attached and feeding. Neither faeces nor scrapings contained vitamin A and the concentrations of carotenoids were almost the same in both but that of β-carotene was higher in the scrapings than in the faeces.

The limpets from Hoy were still alive on arrival and were treated in the same way as the previous group, the faeces being analysed separately. The ovaries of the dissected animals contained no vitamin A and very little carotenoid pigment. The carotenoid concentrations of the rock-scrapings and faeces were very similar, and no conclusion can be drawn regarding possible preferential absorption of carotenoids by the limpet.

TABLE X. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *PATELLA VULGATA* AND ITS FOOD

(All Patella from Harwich, except those of 4. viii. 52 from near Berry Head, Hoy, Orkneys.)

Date	Tissue	No.	Average weight (g)	Oil (%)	Vitamin A		Total carotenoids		β-carotene (µg/g)
					µg/spec.	µg/g	µg/spec.	µg/g	
11. ix. 50	Whole animal	12	7·8	4·7	1·5	0·19	399	51	16
29. i. 51	Visceral mass	22	5·6	2·9	2·3	0·41	208	37	12
	Mantle edge	22	0·2	2·1	0	0	3·9	22	5·9
	Tentacles	22	0·01	5·3	0	0	0·2	21	0
	Total	22	5·8	2·8	2·3	0·40	212	37	11
23. iv. 51	Muscle, foot and mantle	36	2·8	1·2	0·99	0·35	64	23	2·4
	Head	36	0·2	0·9	0	0	3·8	17	4·3
	Digestive gland and kidney	36	1·1	5·7	2·5	2·4	239	226	35
	Intestine	36	0·2	4·9	0·40	2·1	28	142	24
	Ovary	19	1·4	8·5	0·14	0·11	27	19	9·7
	Testis	17	0·8	3·8	0·56	0·67	45	112	30
	Total	36	5·3	3·4	4·2	0·79	398	74	13
24. iii. 52	Head and foot	40	5·9	0·4	0·25	0·04	56	9·6	2·9
	Intestine	40	0·4	2·6	0·28	0·67	21	52	5·4
	Visceral mass	40	1·2	6·2	2·6	2·1	238	192	40
	Testis	18	1·3	0·7	0·40	0·32	76	62	21
	Ovary	22	1·7	4·4	0·11	0·07	25	15	5·0
	Total	40	9·0	1·7	3·4	0·37	364	41	9·5
12. v. 52	Whole animal	13	8·4	2·2	5·2	0·62	479	57	8·8
12. v. 52	Digestive gland	12	1·8	9·6	5·1	2·8	439	239	43
	Intestine	12	0·2	3·9	0·37	1·5	29	121	15
	Rest of body	12	6·3	0·4	0	0	63	10	0·24
	Total	12	8·3	2·5	5·5	0·66	531	64	10
12. v. 52	Faeces	—	2·4	0·7	—	0	—	84	1·9
12. v. 52	Scrapings from rocks	—	7·3	0·3	—	0	—	83	4·8
4. viii. 52	Intestine	20	0·2	6·0	0·06	0·35	25	139	35
	Digestive gland	20	1·0	14	2·5	2·6	272	278	73
	Ovary	10	0·4	3·8	0	0	2·3	5·7	0
	Testis	10	1·0	2·6	0·70	0·74	79	83	12
	Rest of body	20	4·5	0·6	0·45	0·10	59	14	2·3
	Total	20	6·3	3·1	3·1	0·50	395	63	15
4. viii. 52	Whole animal ♀	10	5·4	2·8	2·2	0·40	216	40	7·1
4. viii. 52	Whole animal ♂	10	6·7	2·4	4·4	0·66	383	58	10
4. viii. 52	Faeces	—	0·17	2·8	—	0	—	113	3·5
4. viii. 52	Scrapings from rocks	—	0·2	20	—	0	—	132	Trace

Order Monotocardia

Buccinum undatum Linnaeus

As Table XI shows, two groups of whelks and a group of eggs were analysed. These molluscs were devoid of vitamin A and poor in carotenoids.

Murex trunculus Linnaeus

A singel specimen was analysed with results shown in Table XI. The carotenoids were mainly xanthophylls, although there were traces of β-carotene and possibly astaxanthin.

TABLE XI. OIL AND CAROTENOIDS IN *BUCCINUM UNDATUM* AND *MUREX TRUNCULUS*

Date	Species	Locality	No.	Average weight (g)	Oil (%)	Total carotenoids µg/spec.	Total carotenoids µg/g	β-carotene (µg/g)
. x. 50	*Buccinum undatum*	Burnham-on-Crouch	19	5·9	1·0	34	5·8	0·80
xii. 50	*B. undatum* (eggs)	Burnham-on-Crouch	—	3·81*	0·6	—	2·4	0
. vii. 52	*B. undatum*	Loch Fyne	31	18	0·3	14	0·79	0·07
. v. 54	*Murex trunculus*	Madeira	1	11	2·2	147	13	Trace

Vitamin A absent from all specimens.

* Total weight.

TABLE XII. OIL AND CAROTENOIDS IN *LITTORINA* SPP.

(All specimens collected at Harwich on 11. ix. 50.)

Species	No.	Average weight (mg)	Oil (%)	Total carotenoids µg/spec.	Total carotenoids µg/g	β-carotene (µg/g)
Littorina littoralis	72	210	1·7	9·2	44	14
L. littorea	98	810	4·2	59	73	19
L. rudis	23	61	12	6·1	100	13

Vitamin A absent from all species.

Littorina littoralis (Linnaeus)
Littorina littorea (Linnaeus)
Littorina rudis (Maton)

Results given in Table XII show that these three species all lacked vitamin A. The pigments were xanthophylls and carotenes, with some chlorophyll. In *L. littoralis* β-carotene formed nearly one-third of the total carotenoids. *L. rudis* was richer in fat and carotenoids than its two congeners.

Aporrhais pes-pelecani (Linnaeus)

Table XIII shows the analytical results. The carotenoids included both carotenes and xanthophylls.

Crepidula fornicata (Linnaeus)

Two groups were analysed as shown in Table XIII. They contained good concentrations of carotenoids, both carotenes and xanthophylls.

Cypraea spadicea Swainson

Results for this specimen appear in Table XIII. The carotenoids were mainly xanthophylls.

TABLE XIII. OIL AND CAROTENOIDS IN *APORRHAIS*, *CREPIDULA* AND *CYPRAEA*

Date	Species	Locality	No.	Average weight (g)	Oil (%)	Total carotenoids µg/spec.	Total carotenoids µg/g	β-caroten (µg/g)
13. iii. 52	*Aporrhais pes-pelecani*	Loch Fyne	24	1·13	1·4	2·6	2·3	0
10. x. 50	*Crepidula fornicata*	Burnham-on-Crouch	80	1·42	0·7	24	17	1·3
11. xii. 50	*C. fornicata*	Burnham-on-Crouch	60	1·21	0·7	36	30	1·8
20. v. 53	*Cypraea spadicea*	La Jolla	1	6·1	1·6	49	8·0	0·37

Vitamin A absent from all species.

TABLE XIV. OIL, VITAMIN A AND CAROTENOIDS IN SOME TECTIBRANCHS

Date	Species	Locality	No.	Average weight (g)	Oil (%)	Vitamin A µg/spec.	Vitamin A µg/g	Total carotenoids µg/spec.	Total carotenoids µg/g	β-caro (µg/g
13. v. 54	*Aplysia depilans*	Madeira	2	64	0·3	7·0	0·11	170	2·7	0·32
29. viii. 51	*Clione limacina*	63° 18′ N. 18° 39′ W.	99	0·034	7·6	0	0	3·4	102	1·8
30. i. 55	*Limacina retroversa*	Barents Sea	405	0·00087	1·3	0·008	9·2	0·005	6·0	0

Sub-class Opisthobranchia

Order Tectibranchia

Aplysia depilans Linnaeus

Results for a group of two specimens are given in Table XIV. The ratio of vitamin A ester to alcohol was about 1:5. Both carotene and xanthophylls were detected in the small concentration of carotenoids present.

Clione limacina Phipps

The results for a group of this species appear in Table XIV. The high concentration of carotenoids included both carotene and xanthophylls.

Limacina retroversa (Fleming)

Table XIV shows that this species of pteropod, which was analysed with its shell, was relatively rich in vitamin A, all in the ester form. What little there was of carotenoid pigment appeared from its chromatographic behaviour to be either xanthophyll or possibly astaxanthin ester.

Subclass Pulmonata

Order Stylommatophora

Helix aspersa Linnaeus

Table XV shows the results for a group of garden snails. The pigments were mainly xanthophylls.

Order Basommatophora

Planorbis corneus (Linnaeus) var. *rubra* Oldham.

Results for a dozen of these bright orange snails show (Table XV) that vitamin A was absent and that the concentration of carotenoids was not especially high, the pigment being mainly xanthophyllic, with only traces of carotene.

TABLE XV. OIL AND CAROTENOIDS IN SOME PULMONATES

Date	Species	Locality	No.	Average weight (g)	Oil (%)	Total carotenoids µg/spec.	Total carotenoids µg/g	β-carotene (µg/g)
1. x. 51	*Helix aspersa*	Garden at Shinfield	60	3·9	0·4	6·0	1·5	0·04
5. x. 54	*Planorbis corneus* var. *rubra*	Aquarium dealer	12	2·1	0·3	30	14	Trace

Vitamin A absent from both species.

DISCUSSION

In the lamellibranch species we have examined, vitamin A was found in the mussel, *Mytilus edulis*, the clam, *Mya arenaria*, and the oysters, *Ostrea edulis* and *Gryphaea angulata*, but it was absent from the scallops, *Pecten maximus* and *Chlamys septemradiatus*, and the deposit-feeding bivalve, *Scrobicularia plana*. It was occasionally present in the cockle, *Cardium edule*, and the file-shell, *Lima hians*. Thus in both the lamellibranch orders, the Filibranchiata and the Eulamellibranchiata, there were some representatives with and others without vitamin A and there was likewise no apparent relationship between the carotenoid constituents of the various species and their affinities. All species except *Mytilus edulis* and *Lima hians*, which were richer, had carotenoid concentrations between 1·5 and 20 µg/g, and all species except *Chlamys septemradiatus* contained measurable amounts of β-carotene.

Analyses of the photoreceptive tissues of some of the species showed that vitamin A was absent and so does not appear to be concerned with vision as it may be in Crustacea (Wald, 1941; Fisher *et al.*, 1952). In this connexion it is not possible to determine whether vitamin A in the chiton, *Stenoplax conspicua*, had any visual role, since the animals of this species were analysed whole.

The vitamin A and carotenoid contents of the lamellibranch species do not appear to be associated with the food or feeding mechanism since some ciliary and deposit feeders contained vitamin A and others did not. In those species with vitamin A, *Gryphaea angulata* and *Mytilus edulis* had their richest concentrations in the digestive gland rather than in the rest of the visceral mass, whereas in *Mya arenaria* vitamin A was not invariably present in the digestive gland, and was in all instances absent from that of *Ostrea edulis*. In *Mya* the concentrations of vitamin A were always higher in the rest of the visceral mass than in the digestive gland. The gonads would be included with the rest of the visceral mass in these species and so may have contained the vitamin. This lack of uniformity in the distribution of vitamin A between species indicates no very obvious function for it in the metabolism of these molluscs, in which the vitamin is more probably a chance by-product of metabolism of carotenoids derived from the algae of the food and appearing in those species with suitable enzyme systems. A similar explanation probably applies to the distribution and nature of the carotenoids present.

It is difficult to determine, from their distribution in those lamellibranchs studied, any special functions of the carotenoids present. Scheer (1940) was able, by feeding experiments, to shed some light on the metabolism and role of carotenoids in *Mytilus californianus*. He found that this mussel selected from its food xanthophylls in preference to carotenes and that the gonads acted as storage organs for carotenoids but not for lipids. Our own finding of higher concentrations of carotenoids in the gonads of *Cardium edule* indicates a similar tendency and also a possible reproductive role. In support of his suggestion that carotenoids play a part in the metabolism of the mussel and are not just stored, Scheer observed a reduction in the reserves of carotenoid pigments following feeding on a carotenoid-free diet. It is surprising that he does not mention vitamin A in *Mytilus californianus* since we have repeatedly found it in *M. edulis*. In *M. edulis*, the vitamin A, like the carotenoids, was mainly in the digestive gland, but we do not know whether it is derived unchanged from the food or formed from a precursor. From Scheer's work, conversion seems to be more likely, although vitamin A may not be present in *M. californianus* or essential to its existence, since ZoBell & Landon (1937) have shown that this species can be maintained exclusively on a diet of bacteria and state that Kincaid had found the same to be true for oysters.

Brooks & Paulais (1939) studied the distribution of carotenoids in the oysters, *Ostrea edulis* and *Gryphaea angulata*, and found little difference in carotenoid concentration between the 'green' and 'white' varieties of each species. They showed that the visceral mass in both species contained about twenty times as much pigment as the gills and mantle. We obtained ratios of a similar order and found that most of the pigment was in the digestive gland.

Among the gastropods analysed, vitamin A was present in *Haliotis fulgens*, *Megathura crenulata*, *Osilinus lineatus*, *Patella vulgata*, *Aplysia depilans* and *Limacina retroversa*, but absent from *Astrea undosa*, *Buccinum undatum*, *Murex trunculus*, *Littorina littoralis*, *L. littorea*, *L. rudis*, *Aporrhais pespelecani*, *Crepidula fornicata*, *Cypraea spadicea*, *Clione limacina*, *Helix aspersa* and *Planorbis corneus* var. *rubra*. Of those gasteropods with vitamin A, *Haliotis*, *Megathura*, *Osilinus* and *Patella* are all diotocardian prosobranchs, and *Aplysia* and *Limacina* are opisthobranchs of the order Tectibranchia. *Buccinum*, *Murex*, *Littorina* spp., *Aporrhais*, *Crepidula* and *Cypraea* which all lacked vitamin A are monotocardian prosobranchs. Of the other species without the vitamin, *Astrea* is a diotocardian, *Clione* a tectibranch, *Helix* a stylommatophorous pulmonate and *Planorbis* a basommatophorous pulmonate. Apart from the absence of vitamin A from the single specimen of *Astrea undosa*, all the Diotocardia analysed contained the vitamin and all the Monotocardia lacked it. The Monotocardia are regarded as being less primitive than the Diotocardia, and it may be that in their evolution they have developed an enzyme system capable of breaking down the carotenoids of the food beyond the stage of vitamin A.

The presence or absence of the vitamin does not appear to be related to the food in the prosobranchs since those with vitamin A are all algal feeders, whereas those without it vary from ciliary feeders like *Crepidula*, through algal feeders like the *Littorinas*, to carnivorous predators like *Buccinum* and *Murex*. *Crepidula* has a similar feeding mechanism to that of the oysters and competes with them for the same food in the same environment, and yet the bivalves contain vitamin A. Such a difference would best be explained by the presence of diverse enzyme systems. Two planktonic tectibranchs, the pteropods *Limacina retroversa* and *Clione limacina*, were respectively possessed and devoid of vitamin A, the former being as rich in the vitamin as many of the Euphausiacea, richer in vitamin A than any other invertebrates so far analysed (Fisher *et al.*, 1955). *Limacina* contributes to the diet of plankton-feeding fish and so may be important to them as a source of preformed vitamin A. *Clione*, on the other hand, contained more carotenoids (102 μg/g) than *Limacina* (6 μg/g). Further investigations of the vitamin A content of these and other species of pteropods will be made when material is available to us.

In addition to *Clione*, some species of gastropod, namely, *Osilinus*, *Patella*, *Littorina* spp. and *Crepidula* also had high concentrations of carotenoids, whereas the other species studied had much smaller reserves. Whether these pigments have any positive function cannot be determined from comparison of the different species. We have, however, studied more fully the distribution of both vitamin A and carotenoids in the limpet, *Patella vulgata*. In this species most of the vitamin A (as well as the carotenoids) was in the digestive gland and there was always an appreciable concentration in the intestine, indicating its food origin. Vitamin A was absent from the food, so that *Patella* appears capable of converting a precursor to the vitamin. Comparison of the β-carotene concentrations in the rock-scrapings and in the faeces for the specimens collected on 12 May 1952 (Table X) indicates a possible preferential absorption of this pigment, but the evidence from similar specimens taken on 4 August 1952 is inconclusive or even contradictory, and so judgement must be withheld.

The gonads of *Patella* were of particular interest. They have already been the subject of an intensive study in *P. vulgata* and *P. depressa* by Goodwin (1950) and Goodwin & Taha (1950). These workers found in both testis and ovary β-carotene, echinenone, cryptoxanthin and zeaxanthin in the ratio

5:2:2:2. We found a similar ratio of β-carotene to total carotenoids. Goodwin & Taha (1951) have shown that echinenone and myxoxanthin, an algal carotenoid, are probably identical. They found echinenone in the gonads of *Patella* but we did not observe this pigment in our chromatographs of *Patella* extracts, nor did we see myxoxanthin in the extracts from the rock-scrapings, so that the question of whether echinenone in limpets was obtained by direct passage of myxoxanthin from algae or by conversion was not answered. The much higher concentrations of vitamin A and carotenoids in the testis of *P. vulgata* than in the ovary indicate a difference in the metabolism of carotenoids in the two sexes and possibly a function of the vitamin or pigments in reproduction, although in both ovary and testis the vitamin A was all in the ester (storage) form.

The form of the vitamin A in those molluscs containing it was extremely variable. In *Stenoplax*, *Lima*, *Ostrea*, *Mya*, *Osilinus* and *Limacina* only vitamin A ester was detected, but in *Haliotis* and *Megathura* only the alcohol form was found. In *Mytilus*, *Gryphaea*, *Patella* and *Aplysia* both ester and alcohol were present, but the alcohol form was always in excess. The alcohol is the active form of vitamin A and, in contrast to the condition in Mollusca, vertebrates and Crustacea almost invariably contain an excess of vitamin A ester in the storage organs, although vitamin A alcohol may predominate in the circulatory system of vertebrates.

There seems to be no obvious explanation of the extreme variability in this respect that we have found in the molluscs in relation to their food, ecology or affinities.

We have shown that vitamin A is present in a species of Loricata, some Lamellibranchiata and some Gastropoda, and absent from other species of the second and third classes. It had not previously been reported in either Amphineura or Gastropoda. Those species used as human food contain little or no vitamin A.

We are thus faced with the puzzling finding that some molluscs appear to manage quite well in the absence of vitamin A and its precursors, and it seems difficult to postulate for these substances a function as fundamental in molluscs as in vertebrates. The evidence we present in the paper that follows convinces us that vitamin A is essential for cephalopods in which it may well function in a way similar to that in vertebrates. When it comes to other molluscs, all we can say now is that the presence or absence of vitamin A and its precursors may possibly indicate in them nothing more than the peculiarities of their enzyme equipment.

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SUMMARY

1. Vitamin A, total carotenoids and β-carotene were measured, where present, in one species of Loricata, nine species of Lamellibranchiata and eighteen species of Gastropoda.

2. Vitamin A was found in *Stenoplax conspicua*, *Gryphaea angulata*, *Mya arenaria*, *Mytilus edulis*, *Ostrea edulis*, *Aplysia depilans*, *Haliotis fulgens*, *Megathura crenulata*, *Osilinus lineatus*, *Patella vulgata* and *Limacina retroversa*, but not in *Chlamys septemradiatus*, *Pecten maximus*, *Scrobicularia plana*, *Astrea undosa*, *Buccinum undatum*, *Clione limacina*, *Crepidula fornicata*, *Cypraea spadicea*, *Helix aspersa*, *Littorina littoralis*, *L. littorea*, *L. rudis*, *Murex trunculus*, *Planorbis corneus* var. *rubra* or *Aporrhais pes-pelecani*. The vitamin was found in one of five groups of *Cardium edule* and one of two groups of *Lima hians*. Vitamin A has not previously been reported in Loricata or Gastropoda.

3. In those species containing it, vitamin A was concentrated mainly in the digestive gland or in the visceral mass.

4. With *Patella vulgata* there was evidence that the vitamin A reserves are derived by conversion of carotenoid precursors in the algal diet. In this species the testis contained much higher concentrations of both vitamin A and carotenoids than the ovary.

5. Except in the gonads of *Patella*, there was little evidence that vitamin A has any special function in the molluscs studied. The concentrations of vitamin A were low, except in *Limacina retroversa*, and there was no consistency as between species in the ratios of active to stored forms of the vitamin. The vitamin A and carotenoid contents of any species may possibly reflect its enzymic capacity to break down the carotenoids of the food.

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VITAMIN A AND CAROTENOIDS IN CERTAIN INVERTEBRATES

V. MOLLUSCA: CEPHALOPODA

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(Text-fig. 1)

Introduction

In our previous paper (Fisher, Kon & Thompson, 1956) on Mollusca we pointed out that the Cephalopoda were so different from the other classes in vitamin A and carotenoid relationships that they would be more satisfactorily considered separately. We have so far analysed ten species of cephalopods and found vitamin A in all of them. The studies of previous investigators of vitamin A in these molluscs were confined to its function in the visual cycle and to its contribution to the vitamin A reserves of sperm whales feeding on cephalopods. The carotenoids of cephalopods have been given even less attention than vitamin A.

MacMunn (1883*a*, *b*) was probably the earliest worker to study the pigments of a cephalopod. He examined the alcohol- and ether-soluble pigments of *Octopus vulgaris* and reported that the chromatophores and eyes contained 'tetronerythrin', a substance which probably represented the sum total of carotenoids in the tissues. We have found no further record of similar work on cephalopods until that of Lönnberg (1935) on the carotenoids of *Sepiola scandica*, *Rossia macrosoma* and *Eledone cirrosa*. Extracts from the eyes of all the species contained a xanthophyll and gave a blue colour with antimony trichloride. An extract from the eggs of *Rossia* contained no pigment but gave a strongly blue reaction with antimony trichloride. The remaining tissues of *Sepiola* and *Rossia* contained very little pigment and so were not further investigated. The liver of *Eledone* contained large quantities of carotenoids and gave a greenish blue antimony-trichloride reaction.

In the following year, Verrier & Pannier (1936) first reported the presence of vitamin A in a cephalopod. They stated that they had extracted the vitamin from the visual pigment in the retinas of the eyes of *Eledone moschata*. Escher-Desrivières, Lédérer & Verrier (1938) subsequently examined the pigment from the edges of the pigmented epithelia of the retinas of *E. moschata*, *Sepia officinalis* and *Octopus vulgaris*, and found that it closely resembled vertebrate retinal purples, vitamins A_1 and A_2 as such being absent. Wald (1941) made a quantitative study of the retinas of the squid, *Loligo pealii*, in which he found 1–2 μg of vitamin A_1 and three times this amount of retinene_1 (vitamin A aldehyde) per retina. He found no carotenoids in other tissues of this squid. The amount of vitamin A in the retinas was the same in light and darkness, indicating that the vitamin did not participate directly in the visual process. Wald suggested that squid visual purple was reversibly changed by light into retinene plus protein. Bliss (1948) obtained squid visual purple in an almost pure state, and showed that its properties were very similar to those of vertebrate visual purples.

Further observations on cephalopod carotenoids were reported by Wagner & Vermeulen (1939) and Leong (1939) who detected no carotenoids in cuttlefish, and by Fox & Crane (1942) who studied *Paroctopus bimaculatus* and *Loligo opalescens*. They found only traces of pigment in any of the squid organs but, in the octopus, the liver contained 3·5 mg of carotenoids per 100 g moist tissue and the ink 0·55–0·7 mg/100 g.

Cephalopods had long been considered the most likely source of the rich stores of vitamin A in the liver of the sperm whale, but experimental evidence was first produced by Brachi (1953) who analysed the liver from a 3 ft. long female specimen of *Moroteuthis ingens* taken from the stomach of a sperm whale during the 1951–52 antarctic whaling season. He experienced great difficulty in obtaining a squid free from the nematodes infesting the stomach of the whale, since they immediately attack the liver of any squid eaten. We have found vitamin A in these nematodes, *Anisakis physeteris* Baylis, taken from the stomach of a sperm whale being flensed at the whaling station of Scottish Whalers Ltd in the island of Harris, Outer Hebrides, in 1951. They are the only representatives so far of an invertebrate phylum other than the Arthropoda and Mollusca we have found to contain vitamin A. Brachi also analysed the unsaponifiable matter from squid, *Loligo forbesi*, and cuttlefish, *Sepia officinalis*, caught near Plymouth, but, by his technique, found no vitamin A in them.

MATERIAL AND METHODS OF PRESERVATION

Ten species of cephalopods were collected, some by ourselves and others by marine biologists on research cruises from Plymouth or Millport. They comprised four species of squid, five of cuttlefish and one octopus.

Alloteuthis media (Linnaeus), *Eledone cirrosa* (Lamarck), *Sepia officinalis* (Linnaeus), *Parasepia elegans* (d'Orbigny), *Todaropsis eblanae* (Ball) and some groups of *Loligo forbesi* Steenstrup were collected for us from one of the research ships of the Plymouth laboratory and brought back there alive where they were immediately dissected and the organs weighed and preserved in absolute alcohol.

Several groups of *Loligo forbesi* were collected by a research ship of the Torry Research Station at Aberdeen and dissected by the staff of that station. This material was also preserved in absolute alcohol.

Rossia macrosoma (Delle Chiaje) and *Sepietta oweniana* (d'Orbigny) were taken on various cruises in the Clyde Sea area by the M.V. *Calanus* from the Millport Laboratory. These were dissected and preserved, or preserved whole, after weighing, in absolute alcohol. A single specimen of *S. oweniana* was taken on the surface of Loch Hourn, Inverness-shire, in the bay at Arnisdale, having been attracted by a light lure. This squid was preserved in solid carbon dioxide until arrival at the laboratory at Shinfield, when it was dissected, the organs being weighed and preserved in alcohol.

A single specimen of *Sepiola* sp. was collected near Monaco on 2 February 1952.

Ommastrephes pteropus Steenstrup was caught with a hand-line at two stations of a north Atlantic cruise of the R.R.S. *Discovery II* in November 1954. Only the nidamental gland, eyes and livers were preserved, by immersion in boiling sea water followed by cold-storage. These specimens were weighed when they arrived at Shinfield, and preserved in alcohol.

All the alcohol-preserved specimens were kept in the deep-freeze at −20° C until they were analysed.

CHEMICAL AND PHYSICAL TESTS

Methods

The method of analysis for carotenoids and vitamin A was that described in the first paper of this series (Fisher, Kon & Thompson, 1952). Most cephalopod tissues were much richer in sterols than any material we had previously studied, and in the analyses of extracts from large quantities of tissue these sterols interfered with the Carr-Price reaction for vitamin A. It was necessary, therefore, to remove them. The separation was achieved by extracting the tissues with light petroleum, removing the solvent and dissolving the solids in methanol. The methanol solution was placed for an hour in the deep-freeze at −20° C when most of the sterols precipitated. They were filtered off and washed several times with methanol at −20° C. The methanol was evaporated off from the filtrate and the lipids dissolved in *n*-hexane for the first chromatography.

Since it is broken down during saponification, retinene (vitamin A aldehyde) must be separated from extracts of cephalopod eyes by an additional chromatography. The first chromatography was done as previously (Fisher *et al.*, 1952), but the alumina was weakened with 8% ethanol in *n*-hexane as for the second chromatography. This procedure separated vitamin A ester, which was eluted by 2% acetone in *n*-hexane, from vitamin A alcohol which was eluted by 8% ethanol in *n*-hexane. Retinene was eluted with the ester fraction. This fraction was evaporated down and the residue dissolved in light petroleum (b.p. 40–60° C) for the next chromatography done by the method of Ball, Goodwin & Morton (1948) on a column of Peter Spence activated alumina type 'O' previously weakened by shaking with 10% (w/w) of distilled water. Vitamin A ester was eluted with light petroleum and then saponified and subjected to further chromatography by our usual technique. Elution of the column with a solution of 10% diethyl ether in light petroleum removed the retinene. This fraction was evaporated down, dissolved in *n*-hexane and the absorption spectrum examined in the Beckman quartz photoelectric spectrophotometer.

A sample of pure crystalline retinene, kindly supplied by Prof. R. A. Morton, F.R.S., of Liverpool University, gave in *n*-hexane $E^{1\%}_{1\,\mathrm{cm}}$ 370 $m\mu = 1610$, and this value was used for the calculation of the concentration of retinene from the absorption maximum, after the curves had been corrected for irrelevant absorption by the method of Morton & Stubbs (1946).

Subsequently the retinene solution was again evaporated and dissolved in chloroform for the Carr-Price reaction. The colour in this reaction was read at 664 mμ, 180 sec after addition of the antimony-trichloride reagent. The Thompson (1949) direct reading photoelectric spectrophotometer was also calibrated with the pure crystalline retinene. $E^{1\%}_{1\,\mathrm{cm}}$ 664 mμ for this sample was 4150.

In our earlier analyses retinene was not separated from eye extracts, and where only vitamin A alcohol is reported in these extracts retinene might well have been present initially, but would have been destroyed during saponification.

Results

Order Decapoda

Alloteuthis media (Linnaeus)

A group of six specimens including five females with ripe ovaries, was dissected into the parts shown in Table I. Carotenoid pigments were not present in measurable amounts and vitamin A occurred only in the eyes and ovaries.

TABLE I. DISTRIBUTION OF OIL AND VITAMIN A IN SIX SPECIMENS OF *ALLOTEUTHIS MEDIA*

(Collected near Plymouth on 10. vi. 1952.)

Organ	Average weight (g)	Oil (%)	Vitamin A μg/spec.	Vitamin A μg/g
Eyes (pairs)	0·38	0·9	0·25	0·61
Ovary*	0·44	0·7	0·31	0·71
Liver	0·15	2·3	0	0
Rest of body	7·95	1·2	0	0
Total	8·85	1·2	0·51	0·06

Carotenoids not present in measurable amounts.

* Average values for five animals, see text above.

Loligo forbesi Steenstrup

Five groups of squid were analysed, with the results shown in Table II. In the first group the ratio of vitamin A ester to alcohol in the liver and rest of the body was nearly 3:1, and in the eyes it was 1:11. In those parts with carotenoids, both carotenes and xanthophylls were observed, but no astaxanthin.

In the second group, which was much poorer in vitamin A than the first, the ester:alcohol ratios were, in the eyes, about 1:3, in the liver, about 1:4, and in the rest of the body 6:1. The small amounts of carotenoid pigment in

TABLE II. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *LOLIGO FORBESI*

te	Locality	Organ	No.	Average weight (g)	Oil (%)	Vitamin A μg/spec.	Vitamin A μg/g	Total carotenoids μg/spec.	Total carotenoids μg/g	β-carotene (μg/g)
. 51	Plymouth	Eyes (pairs)	9	7·8	0·9	3·3	0·43	4·1	0·5	0·033
		Liver	9	5·6	11	88	16	34	6·1	0·078
		Ink sac	9	0·6	1·9	0	0	0	0	0
		Rest of body	9	163	1·4	1·3	0·01	41	0·3	0·001
		Total	9	177	1·7	93	0·52	79	0·4	0·005
. 51	Aberdeen	Eyes (pairs)	30	5·0	1·2	0·37	0·07	5·0	0·5	Trace
		Liver	30	4·3	1·8	4·8	1·1	3·1	0·7	0
		Rest of body	30	107	2·0	4·9	0·05	17	0·2	0
		Total	30	116	1·9	10	0·09	25	0·2	Trace
51	Aberdeen	Eyes (pairs)	547	7·5	0·53	1·8	0·24	—	—	—
		Livers	547	7·3	1·1*	7·0	0·96	0·25	0·04	0
52	Plymouth	Ink sac	1	3·6	2·6	0	0	0	0	0
		Eyes (pairs)	1	17	1·3	1·0	0·06	20	1·1	0
		Testis	1	3·4	1·8	0	0	0	0	0
		Gills and hearts	1	20	2·0	0	0	0	0	0
		Alimentary canal	1	13	2·1	2·4	0·18	0	0	0
		Male reproductive ducts	1	16	0·7	0	0	0	0	0
		Liver	1	33	9·0	17	0·52	46	1·4	0
		Caecum	1	47	3·9	11	0·24	74	1·6	0
		Muscle, skin and tentacles	1	830	0·5	5·8	0·01	0	0	0
		Total	1	983	1·0	37	0·04	140	0·14	0
52	Plymouth	Ink sac	14	0·36	2·4	0	0	0	0	0
		Eyes (pairs)	14	5·3	1·1	0·76	0·14	3·5	0·66	0
		Alimentary and reproductive systems	14	3·1	2·7	0·15	0·05	0	0	0
		Caecum	14	1·4	2·8	0·19	0·12	0	0	0
		Gills and hearts	14	3·2	1·8	0	0	0	0	0
		Liver	14	3·6	2·8	0·94	0·26	0	0	0
		Muscle, skin and tentacles	14	100	1·3	0·50	0·005	0	0	0
		Ovaries†	2	31	3·3	13	0·42	0	0	0
		Testis†	1	0·88	0·7	0	0	0	0	0
		Nidamental glands†	2	20	2·4	0	0	9·9	0·50	Trace
		Total	14	117	1·4	4·4	0·04	3·5	0·03	Trace

cluding 1·03 % precipitated as sterols.

† Only three animals with recognizable gonads.

the eyes included both carotenes and xanthophylls, but in the liver and rest of the body only xanthophylls and possibly traces of astaxanthin were observed.

The eyes and livers were dissected from the third group at Aberdeen, preserved separately in abolute alcohol and despatched to us. Portions of the extracts were analysed for vitamin A by physical and chemical methods. Carotenoids were measured in the liver extract, but, inadvertently, not in the eye extract. The bulks of both extracts were retained for biological assay of vitamin A, dealt with later in this paper. The results for vitamin A given in Table II are, as usual, those calculated from the Carr-Price reaction. The value for the eyes was intermediate between those for the two earlier groups and that for the livers was similar to that for the previous lot from Aberdeen. The liver carotenoids were lower in content and concentration than before.

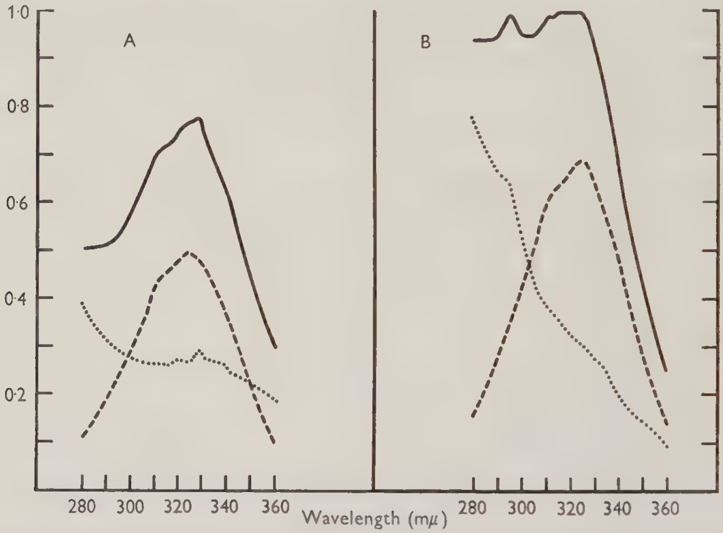

Fig. 1. Absorption spectra of fractions containing vitamin A from non-saponifiable residues of oils extracted from (A) eyes and (B) liver of *Loligo forbesi* (solvent, *n*-hexane). ——, *E* observed; – – – –, *E* corrected; . . . ., irrelevant absorption.

Vitamin A in these two extracts was also examined spectrophotometrically and absorption spectra for vitamin A in the eyes and livers are shown in Fig. 1, with curves corrected for irrelevant absorption by the method of Cama, Collins & Morton (1951). The values calculated from the absorption maximum in (spectroscopic) *n*-hexane at 324 mμ taking $E_{1\,\text{cm}}^{1\%}$ for pure vitamin A alcohol as 1800 were, after applying the correction procedure for irrelevant absorption, for the eyes 2·2 μg per pair or 0·30 μg per g, and, for the livers 5·1 μg per organ or 0·69 μg per g. The large irrelevant absorption, especially at the lower wavelengths, indicates that, despite efforts to remove sterols, a large amount of extraneous materials was still present in the final extracts.

Of the squid collected near Plymouth in June 1952, one was a large male, which weighed nearly 1 kg. It was dissected into the parts shown in Table II. Vitamin A was concentrated mainly in the liver, caecum and alimentary canal, but the amounts were smaller than those found in earlier specimens. The ester:alcohol ratio for the liver vitamin A was 4:1, and for that in the muscle-skin-tentacles extract 1:3. In the caecum the vitamin A was entirely in the ester form. The two components were not separated in the other organs containing the vitamin. The sparse amounts of carotenoids were mainly xanthophylls, although there may also have been a trace of astaxanthin in the liver.

TABLE III. VITAMIN A ESTER, ALCOHOL AND ALDEHYDE IN THE EYES OF *LOLIGO FORBESI* AND *OMMASTREPHES PTEROPUS*

te	Locality	Species	No. of eyes	Average weight (g)	Oil (%)	Vitamin A					
						Ester		Alcohol		Aldehyde	
						μg/spec.	μg/g	μg/spec.	μg/g	μg/spec.	μg/g
i. 53	Aberdeen	*L. forbesi*	100	3·1	1·6*	0·026	0·0086	0·088	0·029	0·77 (0·91)	0·25 (0·30)
ii. 53	Plymouth	*L. forbesi*	67	3·8	1·1†	0·019	0·0050	0·038	0·010	0·96 (0·93)	0·25 (0·25)
i. 54 i. 54	North Atlantic	*O. pteropus*	3	21	0·42	0·57	0·027	0·85	0·040	5·0 (4·1)	0·24 (0·19)

* Including 0·79% subsequently precipitated as sterols.
† Including 0·52% subsequently precipitated as sterols.
Figures in parentheses are values obtained from spectrophotometric measurements.

In the last group, the ovaries were dissected from two females and the testis from one male. The remaining animals were either immature or spent so that the gonads were not recognizable. Other organs were dissected and grouped for analysis in the way shown in Table II. The distribution of vitamin A was as in the male specimen, with concentrations again rather low; carotenoids were almost entirely absent. In the livers the ester:alcohol ratio for vitamin A was 2:9 and in the eyes it was 1:6. In the extracts from the alimentary and reproductive systems and from the muscle, skin and tentacles the vitamin A was all in the alcohol form, but in the caecum it was present only as the ester. The most striking feature of this group of analyses was the large amount of vitamin A, all as the alcohol, in the mature ova.

The possible presence of retinene in squid eyes was more fully investigated in two groups. The results in Table III show that, whereas the vitamin A ester and alcohol concentrations were higher in the eyes of the Aberdeen specimens than in the Plymouth ones, retinene was present in much greater amounts than either of the other two vitamin A components and the concentrations were the same in both groups.

Ommastrephes pteropus Steenstrup

Two specimens of this large squid, taken during a cruise of R.R.S. *Discovery II* in November 1954, were dissected and some organs preserved for us on the ship. The parts we received were the liver, one eye and the nidamental gland from one specimen, and both eyes and the liver from the other. For analytical purposes the two livers were treated separately, but the three eyes were extracted together. The results are shown in Table IV. The livers contained good concentrations of both vitamin A, of which about 75 % was in the ester form, and carotenoids, which were mainly xanthophyllic although traces of astaxanthin may also have been present.

TABLE IV. OIL, VITAMIN A AND CAROTENOIDS IN SOME ORGANS OF *OMMASTREPHES PTEROPUS*

			Average		Vitamin A		Carotenoids	
Date	Organ	No.	weight (g)	Oil (%)	μg/spec.	μg/g	μg/spec.	μg/g
16. xi. 54	Nidamental gland	1	15	2·0	0	0	21	1·4
16. xi. 54	Liver	1	20	9·4	134	6·6	273	13
25. xi. 54	Liver	1	32	11	474	15	1340	42
16. xi. 54 25. xi. 54	Eyes	3	21	0·42	1·4	0·07	31	1·4

β-carotene absent from all organs.

The concentration of vitamin A in the eyes was similar to that found in *Loligo forbesi.* Retinene was also separated from the extract, and the values for the various vitamin A components are shown in Table III. The close similarity in concentration of retinene in the eyes of *Ommastrephes* and in those of the two groups of *Loligo* is very striking.

Todaropsis eblanae (Ball)

Analytical results in Table V show that vitamin A was present only in the liver where the concentration was high; ester and alcohol were not separated in this analysis. The liver was also richest in carotenoids, which were xanthophylls with possibly a trace of astaxanthin.

Parasepia elegans (d'Orbigny)

Results in Table VI show that, as in the previous species, vitamin A was found, entirely in the ester form, only in the liver, but in *Parasepia* the concentration was much lower. Carotenoids were much more widespread in this species and were mainly xanthophyllic. No vitamin A was found in the eyes. It may have been present only as retinene and so destroyed during saponification by our routine analytical technique.

Rossia macrosoma (Della Chiaje)

A single specimen of *Rossia* was dissected into the parts shown in Table VII. The liver was richest in both vitamin A, which was not separated into ester and alcohol, and carotenoids. Small amounts of carotenoids were also found in all other parts.

TABLE V. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *TODAROPSIS EBLANAE*

(Specimen caught near Plymouth on 10. vi. 1952.)

Organ	Weight (g)	Oil (%)	Vitamin A		Carotenoids	
			µg/spec.	µg/g	µg/spec.	µg/g
Ink sac	0·29	2·5	0	0	0	0
Eyes (pair)	9·6	0·9	0	0	8·6	0·9
Alimentary canal	3·7	2·1	0	0	0	0
Male reproductive system	11	1·8	0	0	0	0
Gills and hearts	4·1	1·1	0	0	4·5	1·1
Liver	9·1	11	174	19	70	7·8
Rest of body	111	0·3	0	0	21	0·19
Total	149	1·2	174	1·2	104	0·70

β-carotene absent from all organs.

TABLE VI. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *PARASEPIA ELEGANS*

(Specimens caught near Plymouth on 10. vi. 1952.)

Organ	No.	Weight (g)	Oil (%)	Vitamin A		Total carotenoids		β-carotene (µg/g)
				µg/spec.	µg/g	µg/spec.	µg/g	
Ink sac	14	0·25	0·4	0	0	0·6	2·3	0
Eyes (pairs)	14	1·5	0·9	0	0	2·0	1·3	0
Alimentary canal	14	1·1	1·2	0	0	7·2	6·6	0
Liver	14	1·3	2·5	0·32	0·24	7·5	5·8	Trace
Nidamental glands	11	2·3	1·7	0	0	0	0	0
Ovary	11	2·8	2·6	0	0	2·8	1·0	0
Testis	3	1·1	0·5	0	0	2·0	1·8	0
Rest of body	14	19	0·3	0	0	1·9	0·10	0
Total	14	28	0·8	0·32	0·01	22	0·79	Trace

TABLE VII. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *ROSSIA MACROSOMA*

(Specimen caught in Firth of Clyde on 17. vi. 1954.)

Organ	Weight (g)	Oil (%)	Vitamin A		Carotenoids	
			µg/spec.	µg/g	µg/spec.	µg/g
Eyes (pair)	2·4	0·66	0	0	2·7	1·1
Liver	1·1	5·5	12	11	14	13
Rest of body	21	1·1	0	0	8·3	0·4
Eggs	0·8	21	0	0	3·2	4·0
Total	25	1·9	12	0·48	31	1·2

β-carotene absent from all organs.

Sepia officinalis (Linnaeus)

Three groups of the common cuttlefish, all collected near Plymouth, were analysed with results shown in Table VIII. As in most of the previous species, the liver was the richest in vitamin A and carotenoids, with vitamin A alcohol slightly in excess of vitamin A ester. The carotenoids included both carotenes and xanthophylls. The ovaries contained ripe ova, which were rich in vitamin A alcohol. Vitamin A was present in the alimentary canal as the ester and in the eyes and muscle, brain, skin and tentacles as the alcohol. Next to the liver, the ink sac had the highest concentration of carotenoids, these being mostly xanthophylls.

The eyes were dissected from fourteen much smaller cuttlefish, collected at the same time as the previous specimens, and the analytical results for these are shown in Table VIII. The vitamin A was all in the alcohol form.

As Table VIII shows, the results for specimens collected in November were similar to those taken in June, but the ratio of vitamin A ester to alcohol in the liver was 4:1. As before, the eyes contained only vitamin A alcohol

TABLE VIII. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *SEPIA OFFICINALIS*

(All specimens caught near Plymouth.)

Date	Organ	No.	Average weight (g)	Oil (%)	Vitamin A µg/spec.	Vitamin A µg/g	Total carotenoids µg/spec.	Total carotenoids µg/g	β-carote (µg/g)
7. vi. 51	Eyes (pairs)	2	16	0·67	1·3	0·08	11	0·68	0
	Gills and hearts	2	18	1·6	0	0	18	0·93	Trace
	Ink sac	2	6·6	0·33	0	0	29	4·4	0
	Liver	2	34	4·2	72	2·1	1255	37	2·6
	Female reproductive system	2	49	1·8	0	0	0	0	0
	Alimentary canal	2	19	0·91	3·3	0·18	31	1·6	Trace
	Muscle, brain, skin and tentacles	2	432	0·67	5·6	0·01	77	0·18	0·01
	Ovary	2	57	2·0	100	1·7	0	0	0
	Total	2	632	1·1	182	0·29	1421	2·3	0·1
7. vi. 51	Eyes (pairs)	14	4·4	0·47	0·20	0·05	3·6	0·82	0
16. xi. 51	Eyes (pairs)	2	14	0·60	4·4	0·32	2·2	0·16	0
	Liver	2	34	7·4	67	2·0	210	6·2	0·57
	Caecum	2	6·6	1·8	0	0	0	0	0
	Alimentary canal	2	12	1·3	0·57	0·05	9·1	0·78	0
	Ink sac	2	12	0·46	0	0	8·9	0·74	0
	Ovary	2	1·8	2·4	0	0	0	0	0
	Nidamental glands	2	6·9	1·9	0	0	0	0	0
	Gills and hearts	2	17	0·50	0	0	0	0	0
	Muscle, brain, skin and tentacles	2	406	0·51	0	0	24	0·06	0
	Total	2	510	1·0	72	0·14	254	0·50	0·04
10. vi. 52	Eyes (pairs)	9	2·5	1·1	0	0	3·5	1·4	0
	Ink sac	9	0·76	0·66	0	0	1·5	1·9	0
	Alimentary canal	9	3·5	0·94	0	0	6·4	1·8	0
	Liver	9	4·2	4·2	4·9	1·2	116	28	0·0
	Rest of body	9	39	1·0	0	0	0	0	0
	Total	9	50	1·3	4·9	0·10	127	2·5	0·0

and the alimentary canal only ester. The ovaries were immature and had no vitamin A. Carotenoid concentrations were lower than in the summer specimens.

The smaller specimens in the last group had vitamin A only in the liver, where the ratio of ester to alcohol was about 7:2. The carotenoid concentrations were of the same order as in the June specimens of the previous year.

Sepietta oweniana (d'Orbigny)

Analytical results for the groups and single specimens of this cuttlefish are shown in Table IX. Vitamin A was entirely in the ester form in the first group of specimens. The second group, consisting of three much smaller specimens, was analysed whole, and the results in Table IX show that they were even richer in vitamin A than the first group. Because of the small amount of material, vitamin A ester and alcohol were not estimated separately. In neither of these first two groups were carotenoids present in measurable amounts.

TABLE IX. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *SEPIETTA OWENIANA* AND *SEPIOLA* SP.

Date	Locality	Organ	No.	Average weight (g)	Oil (%)	Vitamin A µg/spec.	Vitamin A µg/g	Carotenoids µg/spec.	Carotenoids µg/g
		Sepietta oweniana							
18. x. 51	Loch Fyne	Whole animals	2	1·3	9·3	4·5	3·4	0	0
9. vii. 52	Loch Fyne		3	0·12	3·1	0·55	4·6	0	0
30. ix. 54	Clyde Main Channel		1	4·3	3·1	5·0	1·1	26	6·0
10. iii. 55	Clyde Main Channel		1	0·93	1·4	3·2	3·5	5·5	5·9
12. iii. 55	Loch Hourn	Eyes (pair)	1	0·32	0·25	0·57	1·8	1·4	4·3
		Liver	1	0·28	0·65	1·4	5·1	1·4	4·9
		Rest of body	1	2·4	0·52	0	0	2·9	1·2
		Total	1	3·0	0·47	2·0	0·66	5·7	1·9
		Sepiola sp.							
2. ii. 52	Monaco	Whole	1	1·4	0·76	0·77	0·54	6·7	4·7

β-carotene absent from all specimens.

The concentration of vitamin A in the large specimen, taken in September 1954, was lower than in the smaller ones but, as in the first group, the vitamin was entirely in the ester form. Measurable amounts of carotenoids were present, but no definite bands appeared on chromatography, so that they were not identified. Another specimen, weighing 0·93 g, was analysed whole. The results in Table IX show that the concentration of vitamin A, all in the ester form, was similar to that in the first two groups. There was again not enough carotenoid pigment for identification.

In the dissected specimen, the liver contained most of the vitamin A, with a smaller amount in the eyes and none in the rest of the body, but the concentration in the whole animal was less than in previous analyses. In the liver there were approximately equal quantities of vitamin A ester and alcohol, whereas in the eyes there was only vitamin A alcohol.

Sepiola sp.

A single specimen was analysed whole with the results shown in Table IX. The small amount of vitamin A was measured only in the ester fraction, since sterols interfered with measurements on the alcohol fraction. There was not enough pigment present in the chromatography for identification of the carotenoid.

Order Octopoda

Eledone cirrosa (Lamarck)

Analytical results for this specimen given in Table X show that, as in the decapods, the liver contained most of the vitamin A. Only the ester was determined. The alcohol fraction gave a red colour in the Carr-Price reaction, probably due to sterols which were not previously removed. These may have interfered in the reaction with any vitamin A alcohol that might have been present. Vitamin A alcohol was slightly in excess of the ester in the eyes. In the other parts of the body vitamin A was all in the ester form, although the presence of sterols may have interfered with the measurement of the alcohol.

TABLE X. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *ELEDONE CIRROSA*

(Specimen caught near Plymouth on 11. vi. 1952.)

Organ	Weight (g)	Oil (%)	Vitamin A		Total carotenoids		β-carotene (μg/g)
			μg/spec.	μg/g	μg/spec.	μg/g	
Eyes (pair)	2·9	0·6	0·59	0·20	5·8	2·0	0
Liver	24	16	64	2·7	340	14	0·20
Hearts, gills, alimentary and reproductive systems	20	0·8	0·54	0·03	46	2·3	0
Rest of body	281	0·5	4·6	0·02	43	0·15	0
Total	328	1·6	70	0·21	435	1·3	0·02

BIOLOGICAL TESTS

Methods

General

The biological activity of the vitamin A fractions from the eyes and livers of 547 squid, *Loligo forbesi* (see p. 68) was measured by rat-growth tests. These were done in the way described by Fisher *et al.* (1952). The standard used was the International Standard for vitamin A at 10,000 i.u./g in cottonseed oil suitably diluted for the tests with arachis oil stabilized with hydroquinone. The material to be assayed was prepared from the non-saponifiable residue after removal of the sterols and extraction of the vitamin A fractions as described earlier in this paper. The residue, containing vitamin A in the alcohol form, was diluted with arachis oil for feeding to rats. During the tests the oils were kept under nitrogen in the refrigerator and their vitamin A content was checked periodically by the Carr-Price test.

Squid-eye oil

Groups of sixteen (all ♀) wholly vitamin A-deficient rats received twice weekly vitamin A standard at the nominal rate of 2 and 4 i.u. daily; a group of fourteen similar rats was dosed at a nominal daily rate of 2 i.u. and another group of thirteen rats at nominal rate of 4 i.u of the test preparation.

Squid-liver oil

As before, the test was done on female rats only. Three groups of eighteen were dosed twice a week with the standard and three with the liver extract at nominal rates of 1, 2 and 4 i.u. daily.

Table XI. Chemical (Antimony-Trichloride) and Biological Measurements of Vitamin A Activity in Squid Oils Fed to wholly Vitamin A-Deficient Rats, in i.u. per gram of Arachis Oil Solution

Organ	Chemical Value	Biological	
		Value	True fiducial limits ($P=0{\cdot}95$)
Eye	195	212	157–279
Liver	210	210	184–241

Results

Table XI shows the results of the two tests. The values for chemical and biological measurements of vitamin A in both eyes and livers show good agreement, indicating that the vitamin was in the all-*trans* form.

Discussion

Although some of the results are unsatisfactory owing to our failure in the early stages of the work to remove sterols from some of the more concentrated extracts and to separate retinene from eye extracts, we believe that they provide some useful information. The supply of specimens suitable for our analyses is so irregular and fortuitous that it may be a long time before we can rectify some of the earlier mistakes. We think it therefore advisable to publish our findings, pointing out results which may be doubtful owing to errors in technique.

We have found in all instances vitamin A in some part of each of the ten species of cephalopod we have studied. The concentration has varied between species, the average being of the same order as the highest found in *Patella*, the richest in vitamin A of other molluscs except *Limacina retroversa* (Fisher *et al.*, 1956).

Except in *Alloteuthis media*, vitamin A was always found in highest concentration in the liver. Usually most of the liver vitamin A was in the ester form, indicating that this organ was acting as a store for vitamin A, as in many vertebrates, but contrasting with the situation in other molluscs where vitamin A in the digestive gland was in the alcohol form (Fisher *et al.*, 1956). In some specimens, mostly mature females bearing eggs, there was less vitamin A ester than alcohol. This fact, coupled with the presence of large concentrations of vitamin A alcohol in the eggs of, for example, *A. media*, *Sepia officinalis* and *Loligo forbesi*, indicates that the liver reserves of vitamin A are used up in the development of the eggs, possibly even to the point of absolute depletion, as in *Alloteuthis* (see Table I), where five of the six specimens contained ripe eggs and the other no gametes at all, probably being spent.

Vitamin A was found in the eyes of all the cephalopods studied, except *Parasepia elegans*, *Rossia macrosoma* and *Todaropsis eblanae*, and it was predominantly or entirely in the alcohol form, although the aldehyde (retinene) was found in those species, *Loligo forbesi* and *Ommastrephes pteropus*, for which the special technique for its separation was employed. When this technique was used for the eyes of *Loligo forbesi* the values we obtained for vitamin A alcohol were about one-tenth of those given by Wald (1941) for *L. pealii*, but the concentrations of retinene were of the same order in both species. It is difficult to reconcile our findings of vitamin A and carotenoids in other parts of *L. forbesi*, especially the liver, with their complete absence, except for the eyes, in *L. pealii*, but, in conversation, Prof. Wald has told us that his investigation, done some years ago, on those parts other than the eyes might well be repeated. According to Brachi (1953), vitamin A was absent from *L. forbesi* and *Sepia officinalis* at Plymouth, but our analytical technique was probably more sensitive than his, normally applied to the more massive quantities of vitamin A found in whale-liver oils.

In the eyes of *Ommastrephes pteropus* the values for both vitamin A and retinene were similar to those found by Wald (1941) in *Loligo pealii*. There are, thus, no qualitative differences between the vitamin A components in the visual cycles of squids living, like *Ommastrephes*, at great depths and those, like *Loligo*, in shallower waters. What is striking is that not only is there a remarkable resemblance between the structures of the cephalopod and vertebrate eyes, as Berrill (1951, p. 200) has pointed out at some length, but also that they function biochemically in a similar way. We have already noted the storage of vitamin A in the cephalopod liver as a vertebrate characteristic, found so far nowhere else among invertebrates, unless the storage of rich concentrations of vitamin A ester in the eyes of euphausiid Crustacea (Fisher *et al.*, 1952) be taken as similar in purpose.

Another interesting resemblance between the vitamin A metabolism of cephalopods and that of vertebrates is indicated by the presence of vitamin A alcohol in ripe eggs, just as found by Neff, Parrish, Hughes & Payne (1949) in hen eggs, where 73–93% of the total vitamin A was in the alcohol form. Parrish, Williams & Sanford (1951) subsequently showed that it was gradually converted to the ester and stored in the embryonic liver during incubation of the chick. No similar observations on developing cephalopod eggs have yet been made. The absence of vitamin A from the ova of *Rossia macrosoma* and *Parasepia elegans* was presumably associated with their immaturity. Lönnberg (1935) reported a positive reaction with antimony trichloride which he described as 'surprisingly strong' for a colourless extract from ripe eggs of *Rossia macrosoma*, which would undoubtedly be due to vitamin A.

From our limited findings the cephalopod groups, squids, cuttlefishes or octopods appear to be fairly uniform in their vitamin A content. The concentrations of carotenoids were comparatively low in all species. In fact, Goodwin (1952) says of the cephalopods: 'The most outstanding fact concerning carotenoids in this group is their comparative absence'. Nevertheless, differences could be detected. The squids were, generally, poorer in carotenoids than cuttlefishes or *Eledone*. This difference may be because they are more active and feed, therefore, on pelagic Crustacea, which usually contain fewer carotenoid pigments than benthic species, very often only astaxanthin being present in the swimming Crustacea, whereas carotenes and xanthophylls occur in other species (Fisher *et al.*, 1952, 1953). We never found astaxanthin in cephalopods in more than faint traces, indicating that it is either little absorbed or is broken down quite rapidly.

Cephalopod livers contained the highest concentrations of carotenoids, but these were extremely variable even within a species. The carotenoids in the livers were usually carotenes and xanthophylls, but carotenes were not found in the livers of *Todaropsis* and *Rossia*. The livers of these two species, and of *Ommastrephes*, did, however, contain traces of astaxanthin.

In a study of the carotenoids of *Paroctopus bimaculatus*, Fox & Crane (1942) found xanthophylls and their esters in the ink. They suggested that this might be a unique method of excreting carotenoids. In some of the species we examined, the ink sacs and their contents were analysed, but not the ink separately. No carotenoids were found in the ink sacs of the squids, *Loligo forbesi* and *Todaropsis eblanae*, but xanthophylls or their esters were present in those of the cuttlefishes, *Parasepia elegans* and *Sepia officinalis*. The ink sacs of the other species studied, including the only octopod, *Eledone cirrosa*, were not analysed separately. We have already pointed out that the cuttlefishes and octopods are richer in carotenoids than the squids, which probably, therefore, do not require any special means of excreting the pigments.

The curves in Fig. 1 show that the absorption spectrum of cephalopod vitamin A is similar to that of all-*trans* vitamin A. The biological activity of squid-liver oil agreed with the potency determined by the Carr-Price test and there is no evidence of a discrepancy such as we found (Fisher *et al.*, 1952) in euphausiid Crustacea between the potencies determined physicochemically and biologically. The presence of all-*trans* vitamin A rather than other isomers shows another resemblance between cephalopods and vertebrates and a difference between them and other invertebrates.

Cephalopods feed mainly on Crustacea, most of which contain vitamin A. As we have already mentioned, the pelagic cephalopods probably feed on swimming Crustacea, among which, because of their swarming habits, the euphausiids must form an important part of the diet. Hjort & Ruud (1929) mention that euphausiids are important as food of the squid, *Gonatus fabricii*, which in its turn forms part of the diet of the bottle-nosed whale, *Hyperoodon rostratus*. Our work on vitamin A in Crustacea so far indicates that the euphausiids are the richest in vitamin A of all the Crustacea (Fisher *et al.*, 1955). They may thus be the main source of preformed vitamin A for the cephalopods which store it and pass it on to their predators, especially the toothed whales. It would be difficult to determine whether these whales eat sufficient cephalopods to obtain all their rich liver reserves of vitamin A preformed, since the feeding rates of whales are unknown. The enormous numbers of cephalopod beaks to be seen attached to the stomach lining of a sperm whale probably represent only a small proportion of the total intake, which may well be adequate.

The cephalopods are the most highly organized of the invertebrates and they exhibit several similarities to the vertebrates and few resemblances to other molluscs in their vitamin A metabolism (Fisher *et al.*, 1956). Perhaps it is more than coincidental that they, the richest in vitamin A of all the molluscs except perhaps certain pteropods, form the main food of the toothed whales just as the euphausiids, the richest in vitamin A of all the Crustacea, form the main food of the baleen whales. Furthermore, the euphausiids are probably one of the principal components of the diet of the cephalopods themselves.

As before, this work was done with financial support from the Development Commission. We are again indebted to many people for their help in the collection of material. At Plymouth, Mr F. S. Russell, F.R.S., and his staff kindly organized the collection of many of the specimens on which we have reported, and at Millport, Mr E. Ford and his staff were as always most helpful. Dr J. A. Lovern of the Torry Research Station, Aberdeen, must be especially thanked for arranging to have squid caught and dissected in such large numbers. Our thanks are also due to Scottish Whalers Ltd. for permission to collect material at their station in the Hebrides. We are grateful to our colleagues, Dr K. M. Henry, for carrying out the biological tests, and Mr P. A. Plack, for determining the absorption spectrum of the sample of pure crystalline retinene, kindly supplied by Prof. R. A. Morton, F.R.S., of Liverpool University. Thanks are due to Miss C. A. Easton for analytical assistance.

SUMMARY

Ten species of cephalopods, namely, *Alloteuthis media*, *Loligo forbesi*, *Ommastrephes pteropus*, *Todaropsis eblanae*, *Sepia officinalis*, *Parasepia elegans*, *Rossia macrosoma*, *Sepietta oweniana*, *Sepiola* sp. and *Eledone cirrosa*, have

been analysed for fat, vitamin A and carotenoids. Vitamin A was present in all species. Carotenoids were found in most species but concentrations were low.

Vitamin A was found mainly as the ester, in largest amounts in the liver, but in females these reserves were depleted by developing eggs, which were rich in vitamin A alcohol. The vitamin was usually present in the eyes, mostly as the aldehyde (retinene) in those species, *Loligo forbesi* and *Ommastrephes pteropus*, in which it was separated, indicating a visual cycle biochemically resembling that of vertebrates.

Cuttlefishes and octopods were richer in carotenoids than squids, probably owing to the more varied and richer carotenoid content in the benthic Crustacea of their diet than in the pelagic Crustacea forming the food of squids. There is evidence that cuttlefishes and octopods excrete excess carotenoids in their ink, whereas squids do not.

Cephalopod vitamin A is probably all-*trans* vitamin A, and there was complete agreement between the potencies, determined chemically and biologically, of vitamin A from squid livers and eyes.

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THE ECOLOGY OF THE TAMAR ESTUARY

VII. OBSERVATIONS ON THE INTERSTITIAL SALINITY OF INTERTIDAL MUDS IN THE ESTUARINE HABITAT OF *NEREIS DIVERSICOLOR*

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(Text-figs. 1–5)

INTRODUCTION

The polychaete *Nereis diversicolor* F. O. Müller is one of the most characteristic elements of the brackish-water fauna of north-western Europe, and inhabits an extremely wide range of salinities. As a background for comparative physiological studies of chloride regulation in worms from different parts of the geographical range, the writer has attempted to describe the salinities and the variations in salinity endured by populations of *N. diversicolor* in ecologically distinct and geographically well separated areas. Three principal environments have been studied:

(1) *Relatively stable high salinity.* Since it was initially hoped to study *N. diversicolor* in a 'marine' habitat, a study was carried out at Kames Bay, Millport, on a presumably marine beach, but it was found that *N. diversicolor* was restricted to a brackish zone produced by underground seepage of fresh water (Smith, 1955*a*). Since I have been unable at any time to find this species except in areas subject to some lowering of salinity, my general conclusion has been that in a 'marine-dominated' habitat *N. diversicolor* behaves as a brackish-water animal and is to be found in the least saline portion of the available ground. A similar conclusion was reached after studies in the Isefjord, Denmark, where the general level of salinity is about 20‰ and quite stable (Smith, 1955*b*).

(2) *Relatively stable low salinity.* The most obvious contrast to the marine-dominated intertidal habitat is found in the inner Baltic Sea, wherein *N. diversicolor* reaches the less saline limit of its ecological range. In these tideless waters the salinity is low and, over most of the year, fairly constant. Studies carried out near the Zoological Station, Tvärminne, South Finland, have also been reported (Smith, 1955*b*). In this region the distribution of *N. diversicolor* is surprisingly unlike that seen in Britain. Whereas in British estuaries the species has been observed to penetrate into nearly fresh water, and much farther than any of its usual brackish-water associates, in the Baltic the reverse is found. The range of *N. diversicolor* is limited at summer salinities in excess

of 4‰, and a number of its brackish-water associates (*Cardium*, *Mytilus*, *Macoma*, *Balanus improvisus*) penetrate into less saline waters. The answer to this paradox may lie in the fact that although the inner Baltic is of fairly stable salinity during most of the year, there is an annual lowering of salinity as a result of melting snows, occurring when temperatures are still very low, and possibly at the breeding period of *N. diversicolor*. Whether the limiting factor is the effect of low salinity upon the adult population (whose osmoregulatory capabilities may be temporarily reduced by low temperature) or upon some phase of reproduction is not yet clear. We must consider, however, that whatever the mode of action, the special hydrographic characteristics of the inner Baltic are important in limiting the spread of *N. diversicolor* into waters of low salinity.

(3) *The estuarine habitat.* In comparison with the two sorts of environment mentioned above, the estuarine habitat is characterized by marked salinity *variation* resulting from tidal action. The present paper discusses this third type of salinity regimen, and completes the series of three studies on the distribution of *N. diversicolor* in relation to salinity which the writer was able to carry out in 1953–54. Most of the observations have been made in the estuary of the River Tamar, with additional observations near the head of the Kingsbridge Estuary.

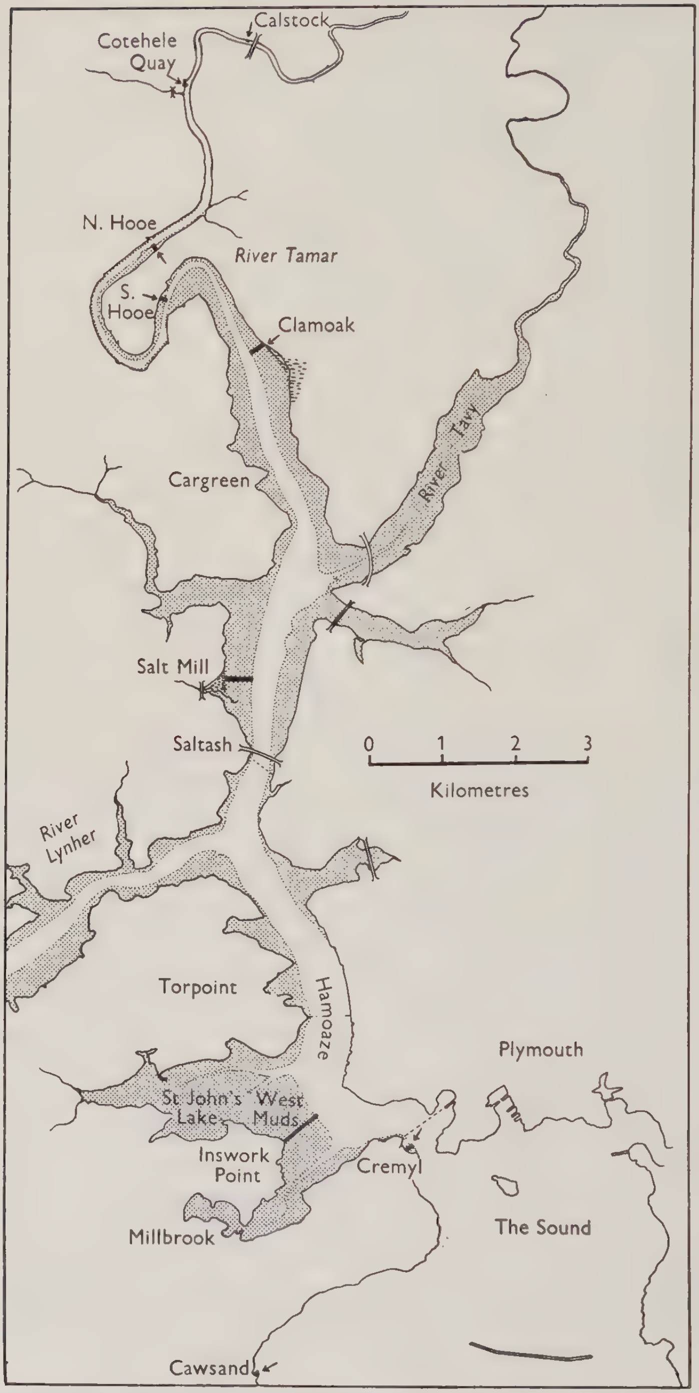
Any estuary exhibits a complex pattern of salinity changes, and individual estuaries may differ very greatly from each other. Day's review (1951) provides a most useful general account, while Rochford's classification of Australian estuaries (1951) illustrates the great diversity which may exist among estuaries in respect to their pattern of salinity variation. In attempting to consider the physiological adaptations of an estuarine animal, we must consider not only mean salinity, which over the range of *N. diversicolor* varies from over 25‰ down to nearly fresh water, but the extent of salinity variation as well. The difficulty of characterizing in any simple way the salinity to which a given population is exposed is very great. At each point within an estuary there may be noted maximum and minimum salinities, but the magnitude of these extremes does not tell the whole story, for their duration and the rates of change of salinity must also be considered (Bassindale, 1943). These factors differ not only horizontally as one proceeds up an estuary from the sea and vertically with intertidal height, but also they vary with time, in all but an idealized estuary where tidal ingressions of sea water were regular from day to day, and freshwater inflow constant and uniform. In actuality, tidal ingressions vary daily throughout the lunar tidal cycle, and are markedly affected by local wind conditions, while freshwater inflow is subjected to seasonal or erratic variation in rainfall. The character of estuaries varies topographically according to volume and configuration of the estuary bed, and with the relative volumes of estuary bed and daily freshwater inflow.

For purposes of the present study, the estuary of the River Tamar has offered the advantages of being relatively accessible from the laboratory at Plymouth, of possessing a long salinity gradient inhabited by *N. diversicolor*, and of being an estuary about which much useful published information is available to the newcomer. Much, however, remains to be learned, and it is hoped that the data presented in this paper will extend the existing knowledge somewhat. Time has not permitted a comprehensive or complete survey, but the effort has been made to base these studies upon reliable previous work, and to relate what has been learned to the total picture.

The most useful introduction to the River Tamar is given by Hartley & Spooner (1938), whose paper should be consulted by any reader not familiar with the locality. By reference to the map (Fig. 1) it may be noted that the lower 10 km of the estuary lies below the confluence of the Tamar and the River Tavy, and that this common estuary is joined by the Lynher River some 5 km from the mouth, which opens into Plymouth Sound. Above the entrance of the Tavy, the Tamar gradually narrows and becomes winding. *N. diversicolor* extends to the vicinity of Calstock (22·5 km from Plymouth Sound), above which point the vegetation and bottom fauna are essentially fresh water, although tidal action extends another 7 km to Weir Head.

The fauna of the Tamar Estuary has been described qualitatively by Percival (1929), whose account is generally useful, although little indication of abundance of individual species is given. It may be noted that Percival worked in a dry summer, and recorded upstream limits for planktonic organisms and certain crustaceans (e.g. *Carcinus* present at Calstock) which may be somewhat atypical. Spooner & Moore (1940) have given a thorough quantitative account of the macrofauna of the intertidal muds of the Tamar as far upstream as North Hooe (18 km). I have been able to add very little general information to their excellent account, except for additional observations on *N. diversicolor* at stations upstream from the limit of their survey. Spooner & Moore reported *N. diversicolor* present scatteringly in St John's Lake, 'apparently here confined to the lower tidal levels', increasing in abundance up-river, and reaching its greatest density at their uppermost station, North Hooe. This increase was believed to be independent of substrate, since equivalent muds are available over more than the entire range of the species in the river. In the lower reaches of the estuary, *N. diversicolor* was found at a lower-intertidal position, but over most of its range was reported to have its maximum density above mid-tide.

The pattern of salinity variation in the Tamar is unquestionably complex, and in comparison with the amount of information available on the fauna, the published data on salinity are still far from complete enough to give an overall picture of the salinity of the *N. diversicolor* habitat. Percival (1929) gave salinity values at or near high and low water for a number of stations from the lower estuary up to the head of tidal action, and the distribution of salinities


Calstock
Cotehele Quay
N. Hooe
River Tamar
S. Hooe
Clamoak
River Tavy
Cargreen
Salt Mill
Saltash
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Torpoint
Hamoaze
Plymouth
St John's Lake
West Muds
Inswork Point
Cremyl
The Sound
Millbrook
Cawsand

Fig. 1. Sketch-map of the Tamar Estuary showing location of stations and points mentioned in the text. Stippled areas represent intertidal muds between H.W.M. and L.W.M. of ordinary tides. Compiled from various sources. A more detailed map is found in Hartley & Spooner (1938).

correlates well with the reduction of the marine fauna as one passes up-stream. Most of Percival's data were obtained during a summer of 'negligible' rainfall (86 mm at Plymouth in 86 days), hence they probably do not represent the lower and perhaps limiting salinities reached in winter and spring. Percival judged from his salinity data that the main body of inflowing sea water did not pass Cargreen (11 km from the mouth) and that the greatest drop in surface salinity took place between Cargreen and the region about Calstock. In this same stretch of river occurred the greatest differences in salinity between high and low tide. Milne (1938) recorded salinity stratification in May–June on one rising and one falling tide in a cross-section of the estuary 7·7 km from the mouth, between Saltash and Neal Point and thus within the wide portion of the river strongly influenced by salt-water ingression (salinity range *c.* 15–32‰). Milne calculated salinity fluctuation at various intertidal heights, and showed that organisms living near low-water mark must suffer the greatest change of salinity at a tidal cycle; those near high-water mark must experience the least change in salinity; those at mid-tidal positions and those living sub-tidally are subjected to fluctuations intermediate in magnitude between those at the first two levels. Additional data for the lower estuary are given, and for a few up-stream points values which are in general agreement with Percival's data, emphasizing the marked drop in mean salinity and the considerable salinity variation of the River Tamar up-stream of its union with the River Tavy. However, the available data are insufficient to define the up-stream limits of *N. diversicolor* in terms of salinity. It has, therefore, been necessary to seek additional data in the region above the middle reaches of the estuary.

METHODS

Field work was carried out from April to mid-June and from late September into December 1954. Since physiological studies on *N. diversicolor* inhabiting the less saline reaches were the main objective, most observations have been confined to the region from Clamoak to Calstock, with comparative observations in the lower reaches (Salt Mill and St John's Lake) as well as in the 'marine-dominated' Kingsbridge Estuary. In the early weeks of work the extent and complexity of the Tamar Estuary were not fully appreciated; in the last few weeks bad weather contributed to incompleteness of the record.

With the limited available time and the difficulty of securing boats when and where needed, it was felt that the usual method of obtaining samples of water close above the mud at representative intertidal levels and at enough different stages of tide would be impractical. The expedient was therefore adopted of measuring the salinity of water contained in the mud itself by means of a series of samples forming a transect of the exposed shore at each station. It is obvious that the salinity of interstitial water reflects in some degree the salinity of the water that has lain above the mud. Precisely what the relationship is between interstitial salinity and the varying salinities of the overlying water is not known. At present it certainly cannot be claimed that the salinity of the interstitial water represents the mean salinity of the overflowing water—it can merely be said that the interstitial salinity must be the resultant of the varying overlying salinities and the duration of the exposure. Until the actual time-course of salinity changes of the water just above a given intertidal point is worked out, it does not appear possible to give a significant 'mean', either arithmetic or harmonic, for salinity at this point. If the change of salinity with time followed a symmetrical pattern (e.g. sinusoidal), the median value would approximate a mean value, but if, for example, the time course followed a pattern wherein one extreme (at high water) acted for a much longer time than the opposite extreme (at low water), then the 'mean' of isolated values taken at high and low water would represent only a median value, and not anything like a true mean. We may assume that the values of interstitial salinity in mud would be subject to the time factor of the salinity variation. We may also assume that under conditions of seasonal variation in the 'mean' salinity at a given point in an estuary—as when the freshwater inflow is elevated during prolonged rains—the salinity of the interstitial water would be shifted in corresponding fashion. But it must be emphasized that no precise measurement of the lability of the interstitial salinity has yet been attempted. The present study was timed, although not intentionally, in such a way that the variability of interstitial salinity with seasonal rainfall has been indicated.

Method of Determining Interstitial Salinity in Mud

The mud of the *N. diversicolor* habitat in the Tamar is soft and usually of a sticky consistency, apparently with considerable admixture of clay. Draining, sucking, or centrifuging water from it is hardly practical. In this survey the simplest methods were adopted, it having been felt not worth while to invest time in more elaborate procedures or equipment until a preliminary survey had indicated the usefulness of such an approach. Samples were taken at low tide at a series of points from the highest level of the mud-flat down to the water-line. The top inch or so of mud was scraped aside, several samples were scooped up with the fingertips from the area thus exposed, and these were packed together in a 175 ml. honey jar, which was filled to capacity to prevent any evaporation. In the laboratory two 30–40 g samples were spooned out from each collecting jar into wide shell vials and weighed immediately to the nearest 0·01 g. Samples were then dried to constant weight in an electric oven at 105 ± 2° C, a process requiring 3 or 4 days. The loss of weight represented the water content of each sample. Into each vial was then pipetted 25 ml. of distilled water, each dried sample was crushed with a small, flat-ended glass rod, the samples were covered to check evaporation, and were allowed to stand for 24 h. The glass pestle was left in each vial, and twice more during the soaking period was used to stir up the contents and to crush any solid lumps. In samples with much clay present this task proved troublesome. Finally, after settling, two 1 ml. aliquots were pipetted from the more or less clear supernatant and titrated with silver nitrate as earlier described (Smith, 1955*a*). From the chloride value obtained on the 25 ml. of added water, and the original water content as obtained by drying, the chloride of the originally contained water was calculated. Salinities were approximated by multiplying the chloride values by 1·81.

The method, as described above, is based upon several assumptions. It is assumed that all samples were taken at about the same depth, roughly the second inch beneath the surface in spots typical of the general area at that level, that no loss of water by evaporation took place before weighing or during the soaking process after drying, that the osmotically active chloride of the originally contained water was quantitatively recovered in the extraction step, and that no such chloride was bound permanently or lost during the drying process. It is further assumed that drying at 105° C did not release constituent water of clay. The method should in theory be unaffected by the actual interstitial water content or by the degree of drainage of the sample, provided no evaporation is permitted. The water-retaining qualities of soils, especially when clay is present, are complex (Baver, 1948), but as far as I have been able to determine, the method described is in principle free of serious theoretical sources of error. In its present form it is admittedly tedious, and, in any future survey, would need to be reduced to a routine to ensure more reproducible results. In particular, a standard method of taking groups of core samples should be employed, and a stricter control of the depth of sample is essential, for we must assume that the salinity of interstitial water in the uppermost layer is more labile than that of deeper-lying water. Further, a standard method of mechanical shaking during the extraction process and a simplified routine for chloride analysis would be necessary before a large-scale survey were undertaken. Present results may indicate whether or not the taking of interstitial salinities is likely to be of value in ecological surveys of estuaries.

Methods of Obtaining Levels

At each sampling station a profile of the exposed shore was determined by means of a dumpy level and a metric surveying staff. Distances of less than 50 yd. were measured with a length of cod line marked off in 5 yd. intervals (always used wet); greater distances were measured by use of the vertical angular adjustment of the dumpy level. The instrument was probably not very accurate for levelling or distance-ranging at distances over 150 m, and its use was not attempted on the wide flats of St John's Lake. In order to assign absolute heights relative to Ordnance Datum, three established Bench

Marks were used. Their heights relative to the newer (Newlyn) datum were furnished by the Ordnance Survey Office:

Location of Bench Mark	National Grid (10 m) Reference		Height above Ordnance Datum (Newlyn)		Height above Chart Datum (Devonport)	
			Feet	Metres	Feet	Metres
South face of west pier of Saltash Bridge to north of ferry landing	SX 4337	5877	6·76	2·06	15·68	4·78
West angle of barn at Clamoak	SX 4378	6421	18·61	5·67	27·53	8·40
South-east face of barn at Cotehele Quay	SX 4236	6803	11·64	3·55	20·56	6·27

At Clamoak and Cotehele Quay, the height of the mud flats could be determined directly. To determine the height of the flat at Salt Mill Creek, markers of the type used by Spooner & Moore, consisting of glass tubes lined with gelatine containing silver chromate and having a capillary at the bottom to damp out wave action, were placed near the Bench Mark at the west end of the Royal Albert Bridge at Saltash. A similar marker was set out on a stake in the mud-flat at Salt Mill Creek. The high-water mark at Salt Mill was thus recorded, and was levelled in relation to the top of the mud flat, top of salting, and to the Admiralty Boundary Stone no. 10, which is conveniently located nearby. The height of this stone is not recorded in Ordnance Survey files, but I have calculated its height, by the method described, as 2·31 m above Ordnance Datum (Newlyn). Returning to Saltash, I related the height of high water as recorded on the indicator to the known height of the Bench Mark, and so was able to assign an absolute level to H.W.M. here and at Salt Mill, and to the profile of the shore at the latter station. Similarly, levels at North and South Hooe were determined with reference to the Bench Mark at Clamoak, and at Calstock with reference to Cotehele Quay. At Calstock and North Hooe the point of high water had to be observed directly, since in December of 1954 salinities above South Hooe were too low to discolour the silver chromate indicator (this difficulty might be eliminated in future by placing a little NaCl solution in each tube before allowing the tide to elevate the water within it). My methods have not differed in principle from those of Spooner & Moore, except that I have used several Bench Marks well up in the estuary rather than a single mark on the shore of Plymouth Sound. The up-river readings were taken at high slack water on windless days, and despite the large freshwater inflow are probably close enough together to rule out error caused by any slope of water in the estuary bed. Like the previous authors, I claim no great accuracy.

Method of Estimating Nereid Population Density

The taking of quantitative worm samples while working alone in the extensive Tamar muds proved difficult, and was attempted only occasionally. At the extremes of the range (St John's Lake and Calstock) only impressions can be given. At Salt Mill, North Hooe, and Cotehele Quay, a metal frame of 0·1 m^2 area was pressed into the mud, the enclosed mud dug out to a depth of about 10 cm, and washed through a pair of sieves of 3–4 and 1·5 mm mesh. Before sieving, the soil was broken apart by hand and as many worms as possible removed intact. The results are inadequate in a quantitative sense but some idea of relative abundance was gained in the effort to be quantitative.

FIELD OBSERVATIONS

Weather

The present study was done partly during May and June and partly in the autumn, mostly under wetter-than-normal conditions. While it is difficult to evaluate the effect of rainfall in a region where local as well as year-to-year variation in rainfall is the rule, it may be noted that May and June of 1954 were months of above-normal rainfall, but followed an unusually dry April, so that the River Tamar was not abnormally swollen, and studies at this time probably reflected conditions not far from normal. However, the summer and autumn of 1954 were, subjectively, among the wettest in local memory, and flooding on the Tamar occurred after a series of autumn storms. Conditions in November 1954 were regarded as severe, and the salinity in the estuary probably approached a minimal level. These subjective estimates are to some extent supported by the rainfall records published in the Monthly Weather Reports of the Meteorological Office. Unfortunately there are no rainfall-gauging stations on the watershed of the River Tamar proper, and it was felt that rainfall at Plymouth or along the lower estuary was less critical than that on the high moors to the north. Accordingly, Table I was compiled from the records for Tavistock and Princetown on the watershed of the River Tavy. The values given are the averages of data from these two stations. Although the records show great variation in

TABLE I

	Rainfall (mm)		
	Average normal	6-year average 1948–53	1954
January	159	153	81
February	147	124	184
March	136	116	167
April	102	107	28
May	88	109	167
June	84	80	157
July	112	112	169
August	137	180	157
September	105	161	201
October	174	158	198
November	188	208	313
December	229	156	164
Totals	1648	1650	1984

a given month from year to year, and from station to station in any given month, the general character of the rainfall in the study area in 1954 is indicated, as well as the 'normal' pattern.

Distribution of Nereis diversicolor *and salinities at selected stations (see Fig. 1)*

'Marine' situations

Careful search was made on each of two occasions in spring at Cawsand and on the shore south of the quay at Cremyl. The substrates consisted of sand among boulders, and at both stations there was clear evidence of some fresh-water seepage. No *N. diversicolor* were found at any intertidal level despite the fact that the substrata appeared in no way unsuitable. At the Plymouth Laboratory *N. diversicolor* is regarded as a mud-dweller rather than a sand-dweller, but at Millport and in the Baltic it is commonly in sand, so that its absence does not appear attributable to a lack of suitable substrate. At no time in the course of over a year's work have I found this species in a situation which could be described as fully 'marine'. Undoubtedly, individuals may be found from time to time in marine water adjacent to populous brackish habitats. Salinities at Cawsand may be assumed to be high; Cremyl may occasionally receive brackish water from the Hamoaze, but no lowered salinities were recorded at this place in ordinary rainy weather. As Spooner & Moore (1940) state: 'In the Plymouth District the status of *Nereis diversicolor* as a marine species is doubtful. Though recorded occasionally from the Sound, it probably never establishes itself in permanent full salinity sea water.'

West Muds (St John's Lake)

Two visits were made in May, and the area from Inswork Point north-west to the channel edge studied at low tide. The exposed muds are here over 400 m wide, and a profile was not attempted. The greater part of the distance from the shore is occupied by an imperceptibly sloping, shell-covered, soft mud-flat, which probably lies close to Ordnance Datum as shown by Spooner & Moore's figure 7 which is a profile of a nearby area. At an estimated 300 m from shore the shelly flat changes to a noticeable slope of finer and even softer mud, which drops to a level very close to chart datum (tide-table level 0·0 ft, or 2·7 m below O.D., Newlyn). Here, at about the mean low-water mark of spring tides (M.L.W.S.T.), the mud slope terminates in a firm bench or flat strip of hard ground overlain by 2 or 3 in. of soft deposit. The fauna of the upper flat is as described by Spooner & Moore; that of the mud slope is far scantier, including mainly *Arenicola marina* and *Nephthys* sp.; that of the hard flat at M.L.W.S.T. is more abundant, including *Sabella pavonina*, *Nephthys* sp., *Melinna palmata* in large numbers, and *Nereis* (*Eunereis*) *longissima*, fairly abundant. In contrast with the findings of Spooner & Moore, I note the following. Large specimens ($>0·7$ g) of *N. diversicolor* occur scatteringly on the outer portions of the shell-strewn flat, apparently below mid-tide level, as the above authors found on the St John's Lake flats (st. B-3, B-5), but I found none on the softer mud slopes, nor on the hard flat at the channel edge. Spooner & Moore report the largest counts of *N. diversicolor* on this 'bare mud...rather firm' area (their stations D-4 at −2·2 m and C at −1·8 m). Since they do not mention *N. longissima*, which is conspicuously present at this level, there is a possibility of a misidentification, regarding which I can draw no conclusions. They also report *N. diversicolor* present at levels as low as −2·0 m in the secondary channel of St John's Lake. This is fully consistent with observations I have made in the Salcombe Estuary near Kingsbridge—there is no question but that *N. diversicolor* may be quite abundant at low-intertidal levels in steep banks of erosional channels in marine-dominated mud-flats (Fig. 5), but I have not found it present in the depositional slopes of very soft silty mud such as occur along the main channel at West Muds. The question of its occurrence on the firmer flat bench at the foot of such slopes is a problem which should be re-investigated. It should be noted that *N. diversicolor* is also found abundantly at high levels along the shore at Inswork Point, in sand black with organic matter, associated with *Cardium*, *Macoma*, and capitellids. Its occurrence here is possibly correlated with freshwater seepage from the land. Samples of water from this sand had salinities of 23·1–30·2‰, whereas the salinity in the main channel on the same day varied from 32·4‰ at extreme low water to 34·4‰ at mid-tide, and was probably even higher at high tide. The population at this upper level, estimated at about 1·0 m above O.D., contained medium and small individuals; it is probably a breeding population, and may be the source of the scattered large individuals on the outer flats. Spooner & Moore consider that salinities below 30‰ are not encountered at St John's Lake even in winter, except possibly at L.W.M.S.T., and Milne's data are in agreement. Apart from the high-level population, which is in a somewhat special situation, *N. diversicolor* seems to occupy a marginal position on the West Muds and seems to be near its seaward limit.

Salt Mill

The transect, at 7·8 km from the Sound, corresponds closely with that worked by Spooner & Moore, and may be levelled with reference to Admiralty Boundary Stone no. 10, whose mark I have estimated as 2·31 m above O.D. (Newlyn). The essential results of observations made on 17 June and 12 October are set forth in Fig. 2. *N. diversicolor* is found in small numbers (but of large individuals) from −1·3 to +0·8 m relative to O.D. (Newlyn). The population does not extend inshore to the edge of the salting, despite the presence of suitable substrate, and its maximum density lies above mid-tide level. Its zonal position is a little lower than that indicated by Spooner & Moore and, furthermore, seems to have a natural upper limit, not set by the actual edge of the mud-flat. The salinity profile indicates a stable situation with no significant seasonal shift; interstitial salinities over the area inhabited by *N. diversicolor* vary only from 25 to 29‰. On 12 October, salinities of the rising surface water were taken as the tide covered the flat. These values (Fig. 2) range from 18 to 25‰, and are lower than those of the interstitial water of the underlying mud. They are also lower than the surface salinities obtained by Milne, in 1937, very near this spot. The low values of the present study undoubtedly reflect the greater rainfall in 1954. The fact that interstitial salinities are higher than those of the advancing water suggests that the former are relatively unaffected by the brief contact with surface water of low salinity during tidal rise and fall, and that at high tide the nereid zone receives water of a salinity greater than 25‰.

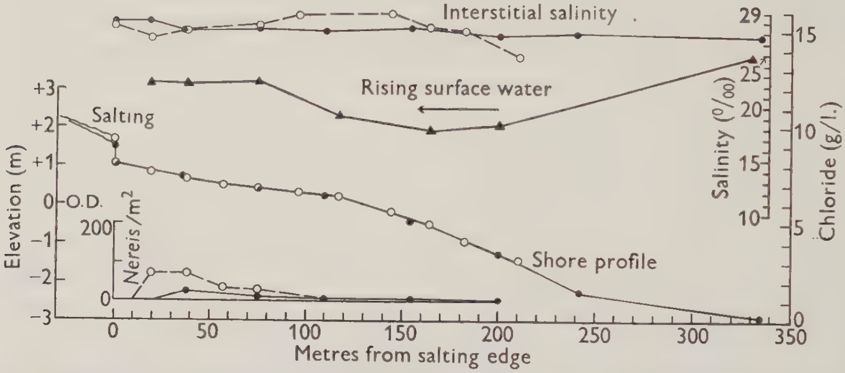

Fig. 2. Salt Mill: upper curves, salinity of interstitial water of mud, below which is a curve (triangles) showing salinity of rising surface water as it covered the flat on 12 October 1954. Middle curve, shore profile. Lower inset, distribution of *N. diversicolor*. Open circles represent values obtained in June; solid black circles, points obtained in October.

Clamoak

This station lies near the upper end of the straight and open part of the estuary, in a region where salinities have started to decline, but where the flats are still quite wide. Saltings are well developed here, 12·5 km from the Sound. *N. diversicolor* is abundant, especially at the upper or inshore levels of the flat and in muddy channels in the salting. It is found from a height of about 0·5 m above O.D. to the foot of the salting at about 0·9 m, and in the channels of the latter to about 1·25 m. No quantitative samples were taken. The salinity profile (Fig. 3A) taken in June showed the salinity across the flat to be as high as that at Salt Mill, except for a pronounced drop near low-water mark. This drop in interstitial salinity coincides with a sharper slope of the outer edge of the mud-flat, suggesting current-scouring of this zone by water of low salinity at low tide. The interstitial salinity of 10 December shows a profound drop to salinities of 5·8‰ inshore and rising to 10·9‰ near L.W.M. This reversal of slope of the salinity profile strongly suggests a salinity stratification such that the higher levels of the intertidal muds are exposed to much lower salinities than the muds near L.W.M. The possibility of freshwater drainage from the adjacent land may well add to a general lowering of salinity near the banks; on 10 December, following prolonged rains, the flat at Clamoak seemed much wetter than usual at low tide, and one had the impression that it was receiving much freshwater drainage from the near-by saltings. A check of the sheet of surface water showed salinities of less than 3‰, while the river water at low tide was as low as 0·4‰. But from the interstitial salinities it is apparent that ingression of salt water even at this period had provided the *Nereis* zone with salinities at least as high as 10‰ at the lower levels and not less than 6–7‰ at the higher levels. In contrast to the lower estuary, this section of the Tamar is clearly one of great seasonal as well as tidal salinity changes, and the salinity profiles of the interstitial water of the exposed shore seem to provide a reasonable picture of the extent of the changes that occurred between June and December 1954.

South Hooe

A station was selected just around the first major bend of the river, 14·8 km from the mouth. The mud-flat is here distinctly narrower than the preceding; the interstitial salinity profile taken in June is lower and drops more sharply toward L.W.M. (Fig. 3B). The December salinity profile shows both a lowering and a reversal of slope quite comparable to and more exaggerated than that observed at Clamoak. *N. diversicolor* is abundant and occupies a wide band vertically from −1·65 m to +1·18 m, O.D. (Newlyn). No population counts were made.

North Hooe

This spot lies 3 km upstream of South Hooe, around the second major bend of the river. At North Hooe the salinity profiles of 6 June and 10 December are still lower, and the seasonal difference is very great, amounting to about 18‰ difference between summer and winter values for the main nereid zone (Fig. 3C). Salinities near the low-water mark are considerably more stable, although not very high: 9–11‰ in summer and 3·5–4·5‰ in winter flood. A count of *N. diversicolor* had been made in May, 400 m downstream, where the mud-flat is somewhat wider. The nereid population was most dense in the upper levels, exceeding 800 per m^2 even before reaching the highest level where the burrows were more numerous than in the spots sampled. A few small *Nephthys* sp. were taken at this point, and dead shells of *Scrobicularia* were seen at North Hooe. Spooner & Moore reported the highest density of *N. diversicolor* at the latter point, where their survey ended.

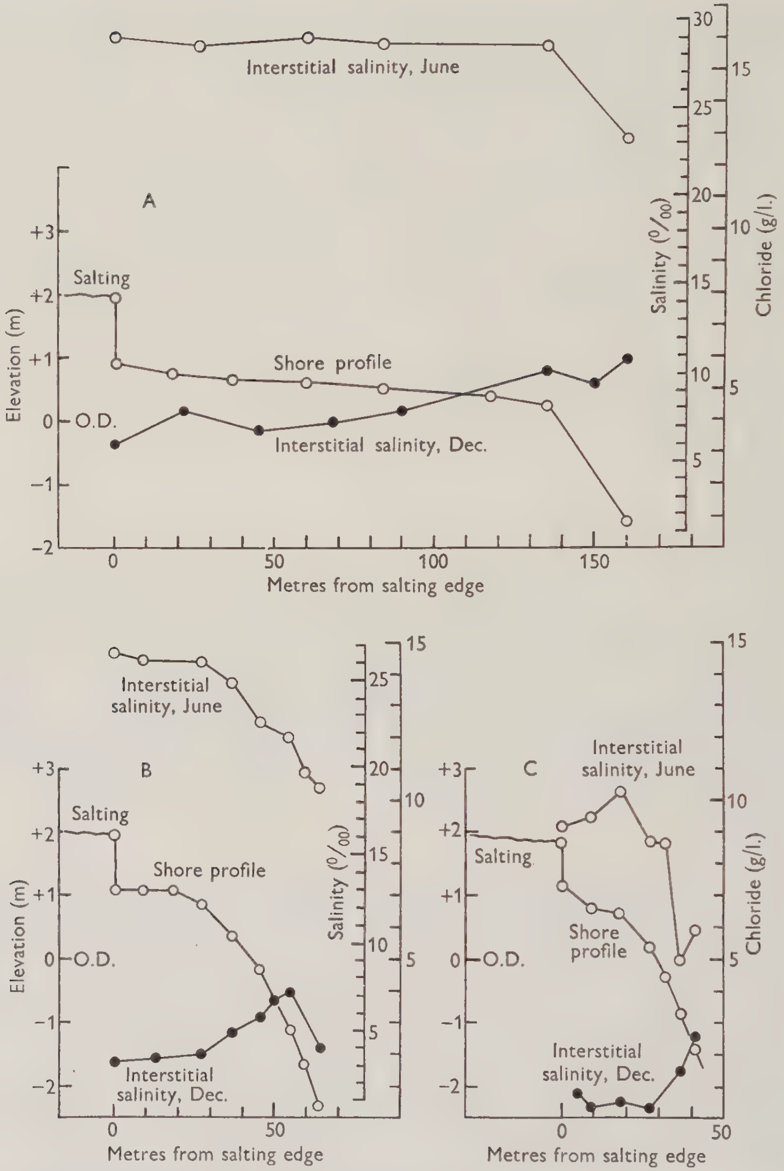

Fig. 3. Shore profiles (June) and interstitial salinity profiles for June (open circles) and December (solid circles). A, Clamoak; B, South Hooe; C, North Hooe.

Cotehele Quay

At this point, 20·5 km from the mouth of the estuary, the river and the bordering mud-banks have become distinctly narrow, and reeds (*Phragmites*) have supplanted typical saltings vegetation. On the stone quay grows a broad band of *Fucus vesiculosus* (det. Dr Elsie Burrows), extending vertically from 1·02 m above O.D. down to 0·52 m below O.D. (Newlyn). In terms of Chart Datum (Devonport) this is from +12·26 ft. to +7·22 ft. tidal height. Percival listed North Hooe as the upstream limit of *Fucus*; this may indicate that the species has spread 2·5 km upstream since 1929, for the growth is extensive enough to have attracted attention had it been growing at Cotehele Quay at that time. Below the *Fucus* is a rich growth of the hydroid *Cordylophora*, typical of oligohaline waters. *N. diversicolor* occurs in considerable density although the available habitat is of small area. Observations were made on a small flat protected from erosion by stone quays (Fig. 4A). The lower part of the steeply sloped mud-bank is poorly consolidated and much gullied. The maximum density of nereids (*c.* 600 per m^2) occurred at the edge of the land vegetation at 1·3 m above O.D., and the population was reduced to scattered individuals at about O.D. level. The population seemed healthy and, judging by the number of small worms present in June, had been breeding. Salinities in the mud were low, varying from 2·6‰ in June to about 0·3‰ in November after a period of flood.

At this time an observation was made which suggested that salinity stratification in these upper reaches of the estuary may be an intermittent phenomenon. Salinity samples were taken at the surface as the tide fell past the zone of *Fucus* (Fig. 4B). A salinity of 0·2‰ was found at high water, when the river lay smoothly, with a gentle downstream drift apparent on the surface. As the tide fell the current gathered strength, a counter-current developed along the quay face where grew the *Fucus*, and the salinity rose to 1·0‰ by 2 h past high tide, by which time the river presented a strong downstream sweep of current. Surface salinity then dropped again until at 3 h past high tide it had reached 0·11‰. The only explanation I can offer is that at high water there is salinity stratification, and that as the ebb develops frictional turbulence is set up, bringing water of higher salinity to the surface. This provides a maximum surface salinity at about the middle of the *Fucus* zone. Later in the ebb the saline water has moved downstream, and the salinity drops sharply. A study of the stability of salinity stratification near Cotehele Quay might prove of interest in connexion with the algal zonation.

At this point, although salinities are generally very low, the tidal and seasonal salinity variation is also nearly minimal. It should be noted that the nereid population at Cotehele Quay, although far denser than any observed in Finnish waters, is living at a salinity of less than half that which characterizes the limit of the species in the Baltic Sea. In the Tamar, as on the south

Finnish coast, both *N. diversicolor* and attached *Fucus vesiculosus* stop together at about the same summer salinity, although this value is much lower in the Tamar than in the Baltic.

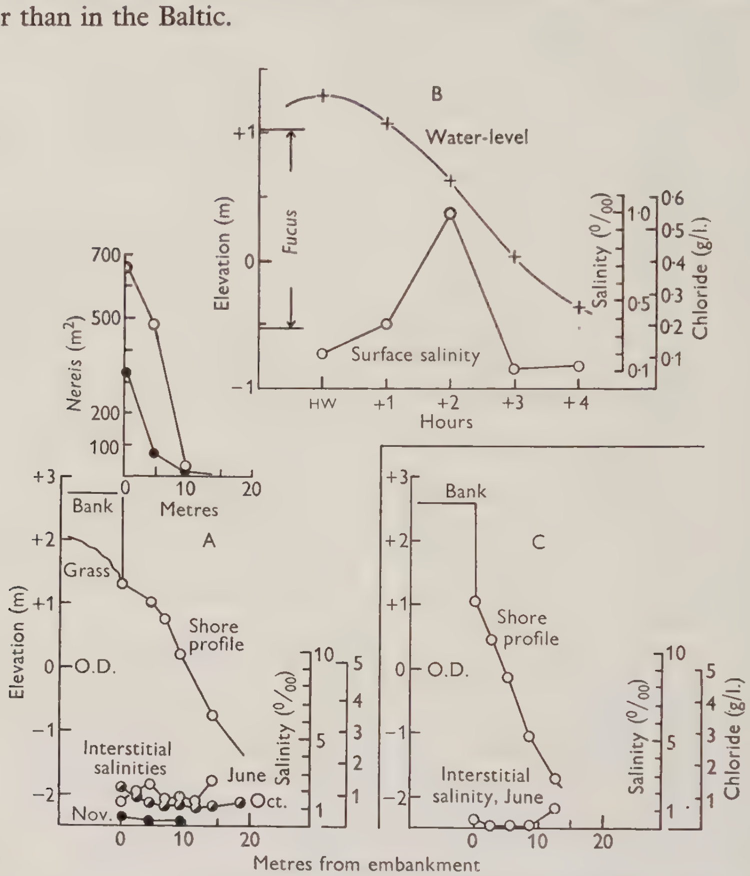

Fig. 4. A, Cotehele Quay: (lower) shore profile and interstitial salinities; (upper) distribution of *N. diversicolor*. B, Cotehele Quay: zonation of *Fucus vesiculosus*. Surface salinities (open circles) during falling tide (height shown by crosses) on 4 November 1954. C, Calstock: shore profile and interstitial salinities, June. Note doubling of horizontal scale in A and C compared with previous figs.

Calstock

Here, 22 km from Plymouth Sound, a few scattered but healthy individuals of *N. diversicolor* were collected on the upper mud slopes just below the railway bridge, always in spots where the mud was of somewhat firmer consistency. The worms were restricted to a narrow vertical band, from O.D. to about 0·5 m higher (Fig. 4C). Associated with them were very abundant tubificid worms, dolichopodid dipteran larvae, and leeches (unidentified) found buried in the mud. Salinities in the mud of the nereid zone were almost nil (less than

0·5‰) but increased to 1·1‰ near low-water mark where the mud was so disturbed as to be apparently devoid of fauna. Percival reported the up-stream limit of *N. diversicolor* as half a mile above Calstock. Careful search at Okeltor, 1 mile upstream, failed to reveal it in a suitable substrate. No other animals or plants of marine affinity were seen at Calstock.

Discussion

The interstitial salinity profiles in Figs. 2–4c show a clear pattern of salinity variation in the River Tamar. If one compares the spring salinities, which were obtained under fairly 'normal' conditions, with those obtained in autumn, three major sections of the estuary can be recognized. (1) A lower estuary, which may be called 'marine dominated', is characterized by wide flats, relatively high salinities, and interstitial salinities little decreased with elevation intertidally and little affected by periods of freshwater flooding. (2) A middle region is characterized by intermediate salinities, with marked variation in salinity with intertidal level, and with a marked lowering of interstitial salinity and a reversal of slope of the interstitial salinity profile at times of freshwater flooding. It may be that excess freshwater discharge is strongly stratified, so that the upper intertidal is more particularly affected; possibly the 'salt-water piston' of the summer is transformed to a deep-lying wedge of lesser volume by the sheer mass of fresh water flowing down above it; perhaps lateral freshwater intrusion from the banks at low tide plays a part. The down-stream limit of this suggested middle estuarine region is perhaps below Clamoak, a point which in summer would seem to be much like the lower estuary but which in winter flood is clearly comparable to points up-stream. The up-stream limit must lie above North Hooe. (3) The upper reaches, characterized by virtual absence of 'flats', show low salinities at all seasons, and little change of salinity either with intertidal level or with season. The down-stream limit in the Tamar is near or below Cotehele Quay. However, these limits cannot be sharp, and it might be best to regard them as entirely labile. If one could imagine a year of low enough rainfall, salinities characteristic of the lower estuary would extend to Calstock, in what we now regard as the upper reaches. Indeed, if we turn not far away, to the Kingsbridge Estuary, we can see this possibility realized.

The freshwater inflow into the Kingsbridge Estuary is so slight, relative to the volume of the estuary bed, that the fauna near Kingsbridge, almost at the very head, is strikingly comparable to that of St John's Lake. A series of observations were made on the flats 1 mile below Kingsbridge (Fig. 5) at a point where the main mud flat is separated from the stony beach by a secondary channel. Because of failure to locate a Bench Mark, the levels shown in Fig. 5 were estimated from the predicted heights of high and low water on 10 October 1954 as corrected for Kingsbridge. This method is particularly subject to error at a point so removed from the sea, and the results may be inaccurate to the extent of perhaps ±0·3 m. The general level of the mud-flat is estimated as close to O.D. (Newlyn) and is thus approximately the same as the flat of St John's Lake as described by Spooner & Moore (1940). *N. diversicolor* was found to be extremely abundant low in the relatively firm erosional slopes of the secondary channel, a position comparable to its occurrence in St John's Lake as reported by the latter authors. In agreement with my findings at West Muds, *N. diversicolor* is also present on the higher shores, where smaller individuals are numerous in muddy sand under rocks up to the upper limit of *Fucus* (+0·6 m), and also in pockets of soft mud held in the remains of hulks on the shore. Along the main channel *N. diversicolor* seemed common on the edge of the flat, but was absent from the soft outer slope and from the firmer bench at L.W.M., quite as at West Muds. The salinity profile (June) is higher than that for Salt Mill, but was not studied after the November rains. There is some indication of a drop in interstitial salinity at the edges of the main flat and in the banks of the secondary channel, which is consistent with the distribution of the estuarine *N. diversicolor*. In general, the salinities at this station were high; the lowest observed was no less than 28‰, obtained in the water of the main channel at low tide in June, and a similar value in the

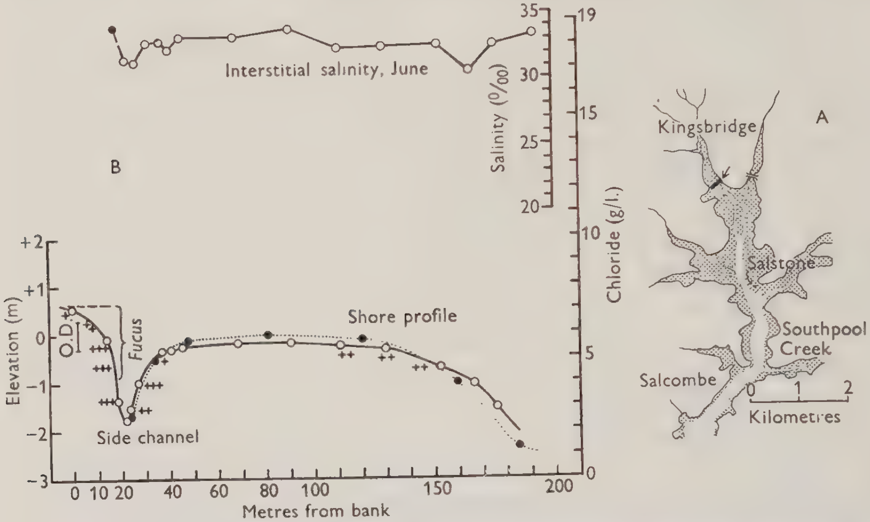

Fig. 5. A, Sketch map of the Kingsbridge (Salcombe) Estuary to show collecting station and points mentioned in text. Stippled areas represent intertidal exposed between H.W.M. and L.W.M. of ordinary tides. B, at top, interstitial salinity profile (June) across flats below Kingsbridge. One point (solid circle) taken in October. At bottom, shore profiles: open circles, June; closed circles, October. Vertical height of *Fucus* zone shown by bracket; estimated relative abundance of *N. diversicolor* shown by groups of crosses. Vertical bar by O.D. represents a possible estimated error in level of ±0·3 m.

secondary channel in October, both at times of fair weather. However, the nearness of the site to the head of the estuary makes it likely that occasional periods of heavy freshwater run-off might effect a greater lowering of salinity than my brief studies encountered.

Lower in the estuary, near the Salstone, *N. diversicolor* is also present, but scarce. A few large individuals were taken in the muddy banks of channels behind the Salstone reef; their position was roughly mid-tidal, comparable to the position observed at West Muds. The species, where found, was associated with the polychaetes *Arenicola*, *Melinna*, *Nephthys*, and capitellids. It was absent from the lower levels inhabited by *Sabella*, *Lanice* and *Eunereis longissima*, and from the very soft mud occupied by *Myxicola*. It is evident that the Kingsbridge Estuary as a whole is, in Rochford's terminology, 'marine-dominated', and only near its head is *N. diversicolor* abundant. Existing reports suggest that this species is scarce in the Kingsbridge (Salcombe) Estuary; Marine Biological Association (1931), following Allen & Todd (1900) reports it numerous only in Southpool Lake, 'in a small gully traversed by a stream of fresh water'. In view of these earlier reports, the abundance of *N. diversicolor* near Kingsbridge deserves mention. As is well known, the marine-dominated Kingsbridge Estuary stands in marked contrast to the Tamar Estuary, which for the greater part of its length is 'gradient-dominated', and characterized by large salinity variations, both spatial and seasonal.

As for the use of interstitial salinities of intertidal mud as indicators of the salinity characteristics of an estuary, it is felt that the results give a picture of what is happening even though the quantitative relationship between salinity in the substratum and the variable salinity of the overlying water is still not understood. The method may be of value to the individual field worker who must sample several localities on the same day, especially if he must travel by land. Certainly less labile than salinities of tidal water, the interstitial salinities are still labile enough to give some picture of changes within past weeks, although how responsive these interstitial salinities actually are, or how stable, remains to be determined. Possibly one could use the surface layer of mud as an indicator of recent changes in salinity, and deeper layers as indicative of general conditions over a longer period. Mud-dwelling animals, by the irrigation of their numerous burrows, may profoundly influence the speed of adjustment of interstitial salinities to variations in the environmental salinity.

Muds represent extremely complex physico-chemical systems (Baver, 1948), of which the detailed composition doubtless varies a great deal within the bounds of a single estuary, so that generalizations as to their water-holding properties should be made with caution. As pointed out by Bourcart & Francis-Boeuf (1942) in their useful discussion of the properties of mud, the chloride values of re-wetted dried samples of marine muds may differ from those based upon the natural water content. These authors make the statement

(p. 27) that the chloride of interstitial water of marine sediments is always higher than that of the overlying water, and suggest that this may be attributable to desiccation during periods of low tide. While this may be a factor in certain estuaries in regions of low rainfall, the intertidal flats of the Tamar would seem as likely to experience lowering of salinity by dilution with rainwater as concentration by evaporation. In situations where, as at Salt Mill, interstitial salinities higher than that of the overlying water are observed, I am inclined to regard this as the result of failure to measure salinity in the lower strata of water at flood tide. In any estuary where salinity stratification is marked, surface salinities will be expected to be lower than interstitial salinities over all but the highest intertidal shores. The presence of a given interstitial salinity at any spot on an intertidal mud slope probably indicates that the spot is bathed by water of at least that salinity at high tide. In certain situations, as described for Kames Bay (Smith, 1955*a*), subterranean intrusion of fresh water may produce local lowering of interstitial salinity, but no instance of this has been seen in the Plymouth area. Instances of intertidal muds retaining a high salinity beneath freshwater streams at low tide are too well known to be cited specifically; they are in no way inconsistent with the general view that the retained interstitial salinities in intertidal muds reflect in general the higher salinities of the tidal water which reaches them.

The chief objective of the present study, to characterize the salinity and the pattern of salinity variation of the habitat of *N. diversicolor* in a typical estuary, requires recognition of the region of greatest abundance of the species. This aspect of the problem has proved beyond the author's capabilities in the field studies described, but fortunately the careful quantitative work of Spooner & Moore (1940) is available and has undoubtedly been applicable in 1954. In Table II their data on the distribution of *N. diversicolor* are summarized, the values being based only upon those stations where the species was actually taken.

TABLE II. DATA OF SPOONER & MOORE (1940)

Location	Range of densities in worms per m²	Mean of densities recorded per m²
St John's Lake	2–56	20
Thanckes Lake	1–350	130
Salt Mill and Ernesettle	4–770	186
Cargreen-Weirquay	8–590	251
Clifton-South Hooe	> 22– > 1020	> 786
North Hooe	400–3030	1715

The data of the present study, treated in the same way, are given in Table III. The total data agree in indicating a maximum abundance somewhere in the region of North Hooe, that is to say, in that part of the river where salinity variation is the greatest. One point of caution should be noted: no data on weight of worms per unit of area are available, and it is notable that where

N. diversicolor is at a high density in the Tamar, the population is of small individuals, rarely exceeding 0·15–0·20 g. On the other hand, the scattered worms at Salt Mill and St John's Lake are mostly of very large size. Dales (1951) has pointed out that a population of *N. diversicolor* may have a low percentage of males, not over 10%, and that these must be sought out by the females if reproduction is to be accomplished. Females ordinarily spawn and die at an age of 1 year or less. However, those females which fail to find a male may survive for 18 months or perhaps 2 years, and grow to very large size.

TABLE III. DATA OF THE PRESENT STUDY

Location	Range of densities in worms per m²	Mean of densities recorded per m²
Salt Mill	3–70	28
Clamoak	(Abundant)	—
South Hooe	(Very abundant)	—
North Hooe	45–>800	436
Cotehele Quay	5–660	204
Calstock	(Occasional)	—

The occurrence of scattered large worms may thus be indicative of a non-reproductive population, perhaps rendered so by such wide spacing that encounters between the sexes are unlikely. On this assumption, the large worms at St John's Lake and Salt Mill would not, despite the high weight per unit area they represent, be considered as anything but a marginal population. It must be added, however, that large worms may occur in quite dense populations in mud, as at Millbrook Lake and near Kingsbridge, and the problem requires clarification. In respect to the River Tamar population of *N. diversicolor*, the optimal region seems to lie up-stream from the region reached by the main mass of tidal sea water, and to centre in the region of maximum salinity variation.

In summing up the series of studies, of which this article is the third, on the distribution of *N. diversicolor* in relation to salinity, it is well to point out that no simple relationship has been detected. We have seen (Smith, 1955*a*) that the apparent zonation of the species on the sandy beach at Kames Bay, Millport, is correlated with a zone of lowered interstitial salinity. This may in part be the result of abundance of *N. diversicolor* in areas where less interspecific competition is encountered. A clearer example of this sort of limitation is seen in the Isefjord (Smith, 1955*b*), where the apparent restriction of *N. diversicolor* to zones of very low salinity is the result of its exclusion from a large part of its former range as a result of competition and/or predation by another species of nereid. In the Tamar, *N. diversicolor* is most abundant in areas which other polychaetes are unable to utilize, but it is also abundant in the more saline head of the Kingsbridge Estuary where competition by other, more marine, species must be severe. Interspecific competition is perhaps not a factor of over-riding importance in limiting the distribution of *N. diversicolor* in the Plymouth region, although it may be so in certain situations elsewhere.

Tidal factors *per se* are also difficult to evaluate, but are probably of secondary or indirect importance. *N. diversicolor* is characteristically an upper mid-tidal form in the Tamar, but can occupy lower positions, as it does in the banks of secondary channels in St John's Lake and near Kingsbridge. Here we find a slightly firmer substrate, as well as somewhat lower salinities, than in adjacent higher areas. Over most of its range in the Tamar, *N. diversicolor* is characteristic of the 'flats', which, as noted by previous workers, show a relatively greater area of intertidal mud above mid-tide level as one goes upstream (Hartley & Spooner, 1938). The level of the saltings which border the upper Tamar at many points is said to be quite constant. The pertinent data on heights of mud-flats and saltings obtained in the present survey are given in Table IV.

TABLE IV. HEIGHTS IN METRES ABOVE O.D., NEWLYN

Location	Top of salting	Highest level of mud	General level of 'flat'
Calstock	(Embanked)	1·04	0·1 to 0·5
Cotehele Quay	(Embanked)	1·30	0·7 to 1·3
North Hooe	1·83 to 1·88	1·15	0·7 to 1·0
South Hooe	1·85 to 1·90	1·18	1·13 to 1·15
Clamoak	1·93 to 1·96	0·90	0·40 to 0·75
Salt Mill	1·50 to 1·70	1·05	0·25 to 0·8
St John's Lake (from Spooner & Moore)			−0·2 to +1·0

These slight figures suggest a constant level of about 1·8–2 m above O.D. for the tops of saltings (the lower salting at Salt Mill may be eroding away). Also fairly constant is the highest point of the mud (i.e. the foot of the salting cliff) at about 1 m above O.D. The general level of the main flat is not precisely determined, but shows an increase in level from the lower estuary up to the first great bend in the river at South Hooe. Above this point, with the narrowing and steepening of the intertidal mud, the 'flats' themselves seem to become more inclined, and the outer edge lower, possibly as the result of current scouring.

At Cotehele Quay and up-stream, flats are almost indefinable, and since at both Cotehele and Calstock the measurements are at spots disturbed by man-made embankments, no conclusions should be drawn. It would seem that the somewhat lower intertidal position of *N. diversicolor* in the lower reaches of the Tamar Estuary reflects the general level of the suitable substrates. This situation may provide the incidental advantage that in the upper reaches of the estuary *N. diversicolor* is placed above the very low salinities of the ebb-tide, and in the marine-dominated lower reaches is exposed to favourably brackish water at low tide. It should be noted that if salinity stratification occurs in the middle or upper reaches of an estuary, as seems to happen in the Tamar, the upper intertidal and the lower intertidal zones are exposed longer to surface waters of low salinity than the mid-tidal levels, over which the rising or falling surface water passes most rapidly. That exposure at low tide is of direct advantage to *N. diversicolor* would seem unlikely except as it may reduce competition from less exposure-resistant forms. Submergence *per se* has not been shown to be deleterious to *N. diversicolor*, and this species must live permanently submerged in its habitat in the Baltic Sea. Only by considering the several factors of substratum, intertidal position, pattern and degree of daily and seasonal salinity variation, temperature, interspecific competition, and so forth, can the relation of a species to any one factor such as salinity be fully appreciated. The further problem of whether populations of a species in different parts of a wide geographical range are physiologically distinct in their responses to the factor in question should be viewed against the background of the ecology of the species. Certain information on the comparative physiology of *N. diversicolor* in response to low salinity will be presented in a later paper (Smith, 1955*c*).

This study was made possible by a Fulbright grant to the writer as an Exchange Lecturer in Zoology at the University of Glasgow. My work at the Laboratory of the Marine Biological Association, Plymouth, was greatly aided and made pleasant by innumerable kind and helpful acts by the staff, scientific, technical, and administrative, of the Laboratory. Since I cannot thank them individually, I wish to express to the Director, Dr F. S. Russell, my appreciation of what he and his staff have done for me. I am also grateful to my wife for the thankless task of assisting in the surveying of mud-flat levels.

SUMMARY

The estuarine habitat of *Nereis diversicolor* in the River Tamar has been surveyed, and the distribution of this worm related to salinities in a 'normal' spring and a rainy autumn.

The method of evaluating the salinity at given points within an estuary by means of 'salinity profiles' of the interstitial water of the intertidal mud is put forward, and the results of the method shown to be informative and consistent with the results of previously used methods.

N. diversicolor is found to reach its maximum population density in that portion of the Tamar Estuary where the greatest salinity variation, both seasonally and with intertidal height, is the rule. At its up-stream limit it regularly endures salinities of less than 0·5‰.

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ON A NEW SCYPHOMEDUSA, *PARAPHYLLINA RANSONI* N.SP.

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(Plates I and II, and Text-figs. 1–3)

Maas (1903) described two specimens of a deep-water coronate scyphomedusa collected on the Siboga Expedition in the Malay Archipelago. He erected for these a new genus *Paraphyllina* and called the species *P. intermedia*. It differed from *Periphylla* in that the rhopalia were perradial and not interradial. In 1903, Lo Bianco figured a specimen taken in the Mediterranean near Capri which was identified for him by Maas as *P. dodecabostrycha* (see also Maas, 1904, p. 48, footnote); Mayer (1910) saw this specimen and re-described and figured it as *Paraphyllina intermedia*. A fourth specimen ascribed to this species by Ranson (1936) was found washed up on the shore at Villefranche.

All these specimens were the same in their essential characters, but differed in some details, especially in the form of the gonad and in the coloration.

In a deep-water haul with the 2 m ring trawl made by R.V. *Sarsia* on 28 April 1955 with 500 fathoms of wire out off the mouth of the English Channel in 48° 26′ N., 9° 42′ W. some four dozen scyphomedusae were caught which belonged to the genus *Paraphyllina*. Most of the specimens, which ranged from 11 to 35 mm in diameter, were in an excellent state of preservation.

These specimens agreed most closely with the medusa described by Ranson, and at first I was inclined to regard them as *P. intermedia*. Fortunately Dr P. L. Kramp suggested to me that the two original specimens described by Maas might still be in existence. On inquiry they were found to be in the Zoölogisch Museum at Amsterdam, and Professor H. Engel most generously sent these two type specimens to me to see.

Examination showed at once the adequacy of Maas' description, and that my specimens were not *P. intermedia*, and must therefore be regarded as a new species. As they are undoubtedly the same as the medusa described by Ranson (see footnote on p. 110), I have great pleasure in naming this species *Paraphyllina ransoni* after Gilbert Ranson, whose researches have added much to our knowledge of medusae.

Paraphyllina ransoni n.sp.

Umbrella with hemispherical dome-shaped summit, with deep corona groove situated somewhat nearer to the umbrella margin than to the summit, with fairly thick jelly, and with rectangular pedalial thickenings separated by deep furrows where the sixteen septal attachments of the upper and lower sides of the umbrella run. Rhopalial pedalia slightly narrower than the tentacular pedalia. Well-developed, continuous, coronal muscle band on subumbrella surface. Sixteen lappets with evenly rounded margins; the lappets on either side of the rhopalia are each a little more than two-thirds the width of those of the intertentacular pairs, and somewhat shorter on their rhopalial margins. Twelve solid marginal tentacles, eight adradial and four interradial; each about the length of the radius of the umbrella. Four perradial rhopalia, each with hood, statocyst and pigmented bulb, but no ocellus with lens. Eight adradial flattened elongated gonads, each forming an asymmetrical W whose interradially situated arms are coiled inwards and whose adradial arms bend outwards towards the perradii.

Stomach wall attached interradially to umbrella dome over four flattened triangular septal plates giving rise to four deep triangular pockets externally. Eight rows, or phacellae, each of about twenty or more simple gastric filaments, one along each side of the triangular septal plates. Four perradial horizontal entrances from stomach cavity into gastrovascular sinus, which is divided by the sixteen septa; each septum running from just above coronal muscle band to half the length of the marginal lappet, the rhopalial septa being slightly the longer. Manubrium slightly folded and reaching about to bases of marginal tentacles with four perradial thickenings and very slightly crenulated margin. Whole subumbrella and marginal tentacles uniformly coloured deep chocolate red,[1] darkest in stomach but reaching right to umbrella margin; gonads colourless. Size up to 35 mm in diameter (25 mm at coronal groove).

FURTHER DETAILS

Stomach. Each of the gastric filaments, which number about 160 or more in all, arises singly; very occasionally two may arise from one root. There are sometimes two or three terminal filaments at the apex of each triangular septal plate, and occasionally an isolated tuft of filaments on the stomach ceiling, presumably left behind during the downward movement of the areas of attachment as the umbrella grows.

Gonads. The W-shaped form of the gonads is very characteristic. Ranson's description of the gonads in his specimen agrees well with mine except that he said that each divides into three branches interradially. From Ranson's drawing the gonads have the appearance of being very fully developed.

In some of my specimens with most fully developed gonads the interradial areas of the W may recoil upon themselves and overlie or even fuse with their neighbours, so that their true outlines are not very clear, or they may be abnormal in shape.

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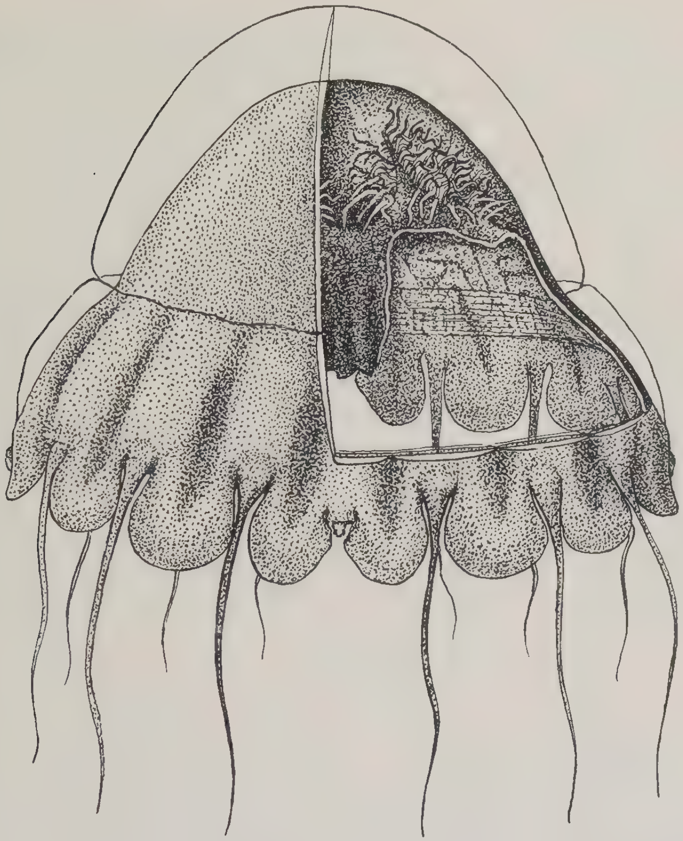
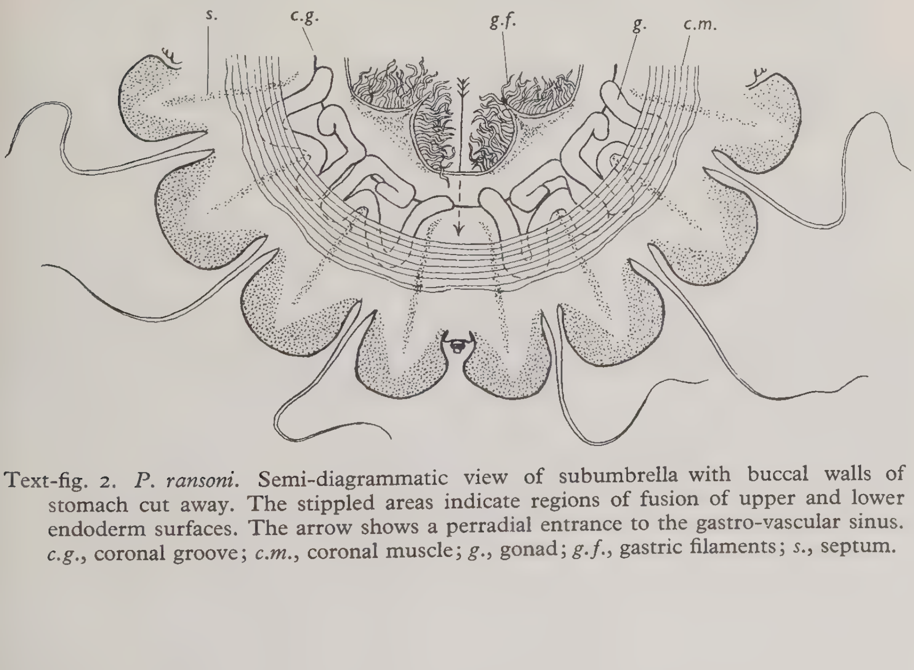
[1] Specimens preserved in formalin and sea water.



Text-fig. 1. *Paraphyllina ransoni.* Adult medusa with a section of the umbrella and a portion of the manubrium cut away to show the subumbrella and internal anatomy.



Text-fig. 2. *P. ransoni.* Semi-diagrammatic view of subumbrella with buccal walls of stomach cut away. The stippled areas indicate regions of fusion of upper and lower endoderm surfaces. The arrow shows a perradial entrance to the gastro-vascular sinus. *c.g.*, coronal groove; *c.m.*, coronal muscle; *g.*, gonad; *g.f.*, gastric filaments; *s.*, septum.

In one of my smallest specimens the shape of the gonads approached that shown by Mayer in his figure of Lo Bianco's specimen. They were still crescent-shaped, and had not assumed the form of a W which is brought about by the extension of the gonads as they grow towards the umbrella margin on either side of the septa. In practically all specimens, even the smallest, the typical shape of the gonad had already been assumed.

In one of the male specimens it appears that the fully developed gonads may have started to degenerate. Four of the gonads are reduced to crescent-shaped strands of tissue (Pl. II, fig. 2). Two gonads are complete, but the remaining two have been reduced to small pieces (Pl. II, fig. 1).

In female gonads the ova are in different stages of development (Pl. II, fig. 4); in the males the spermatozoa are developed in elongated, oval or slightly branched seminiferous follicles arising from the gonad wall (Text-fig. 3*d*).

The gonads are attached to the upper wall of the gastrovascular sinus along one edge.

Coloration. In general coloration my specimens were similar to that described by Ranson. The colourless transparent thickened dome of jelly above the coronal groove and the transparent pedalia are striking features.

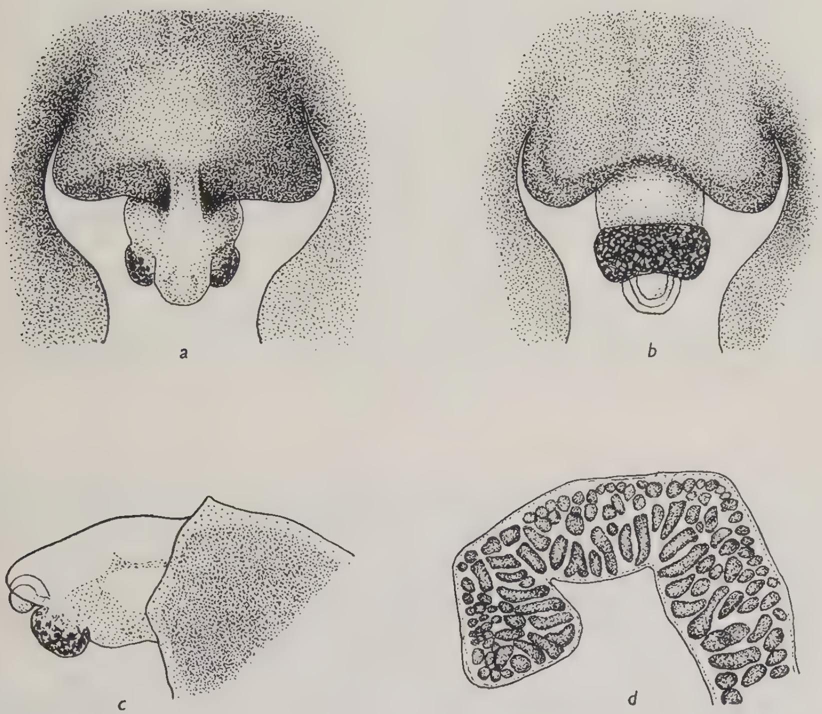
Sections show that over the main body of the medusa the coloration is limited to the mesogloea lying immediately beneath the endoderm; here it forms a dark membranous layer which fades off gradually, but usually for only a short distance, towards the exumbrellar surface (cf. Fox & Millott, 1954, p. 398 and fig. 6 in *Pelagia*). In the buccal walls of the stomach the mesogloea is coloured brownish yellow throughout, and is especially dark near the base; in these stomach walls the coloration may also be dense just beneath the outer ectodermal epithelium. The mesogloea of the marginal lappets is coloured throughout, and the thin dark zone is to be found here both on the exumbrellar and subumbrellar sides. In the marginal tentacles the coloration is also in the mesogloea.

The gonads are colourless, and appear white in contrast to the surrounding coloration. In fresh specimens the gonads cannot be seen through the exumbrella owing to the dark coloration; but after seven months in formalin they were clearly visible, the whole medusa having become much more transparent.

Marginal sense organs. The rhopalium consists of an upper short transparent spoon-shaped hood, covering the stalked statocyst, which is enveloped on the subumbrellar side by an opaque pigmented bulb in the form of a collar; this bulbous collar has ectodermal pigment. The hood and bulb fuse to form the main stalk of the rhopalium, the lower half of which is wide and rounded and the upper half of which narrows to a thin carinate crest. At its base the rhopalium joins a wedge-shaped cushion-like thickening of the umbrella margin, which has the typical mesogloeal coloration.

Text-figs. 3*a–c* show the general structure of the rhopalium, and a photograph of a radial section is given in Pl. II, fig. 3. It is to be noted that the pigment of the bulb is definitely on the outside in the ectoderm, and there is no ocellus with lens such as Maas described in *P. intermedia*.

Umbrella margin. In a few of my specimens the edges of the marginal lappets are undamaged, but in most they are somewhat frayed (see Pl. I, fig. 1).



Text-fig. 3. *P. ransoni.* *a, b, c*: exumbrellar, subumbrellar, and lateral views respectively of rhopalium (*a, b*, reflected light; *c*, transmitted light); *d*, portion of male gonad.

Above the bases of the marginal tentacles and rhopalia there are six to eight, or more, radially directed dark stripes, typical of many coronate medusae. Sections show that these are thickenings of the mesogloea lying under the coronal muscle, which probably play a part by their elasticity in the contraction and expansion of the umbrella margin. They are clearly shown in Pl. I, fig. 2.

Size. The approximate total diameters of the medusae lying free in the formalin and sea water were as follows:

Diameter (mm)	11–14	15–19	20–24	25–29	30	35
No. of specimens	3	13	14	8	2	2

The differences between *P. intermedia* and *P. ransoni* may be summarized as follows:

	P. intermedia	*P. ransoni*
Colour	Limited to part of stomach	Over whole medusa
Gonads	Bean-shaped: above muscle ring	W-shaped: extending under muscle ring
Rhopalium	With ocellus with lens	Without ocellus with lens

It is now known[1] that the medusa described by Ranson is *P. ransoni*, but I should not like to say to which species Lo Bianco's specimen belonged; in its coloration it fits *P. intermedia* but it differs from *P. intermedia* in the shape of its gonad.

Specimens from this collection have been deposited in the British Museum (Natural History) and the holotype has been given the registered number B.M. 1955.11.2.1. Specimens have also been deposited in the Universitetets Zoologiske Museum, Copenhagen, the Zoölogisch Museum, Amsterdam, and the Muséum National d'Histoire Naturelle, Paris.

My thanks are due to Capt. C. A. Hoodless and the crew of R.V. *Sarsia* who collected the specimens; especially to Dr J. S. Alexandrowicz for much help and for making the sections and preparations illustrated in Pls. I and II; to Dr D. P. Wilson for the photograph in Pl. I, fig. 1; and to Dr P. L. Kramp for checking the description against the original specimens.

SUMMARY

A number of specimens of a new coronate scyphomedusa of the genus *Paraphyllina* Maas were caught in deep water off the mouth of the English Channel in April 1955.

A detailed description is given, and the species has been named *Paraphyllina ransoni* n.sp.

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[1] Dr Ranson has confirmed this identity after seeing two of my specimens.

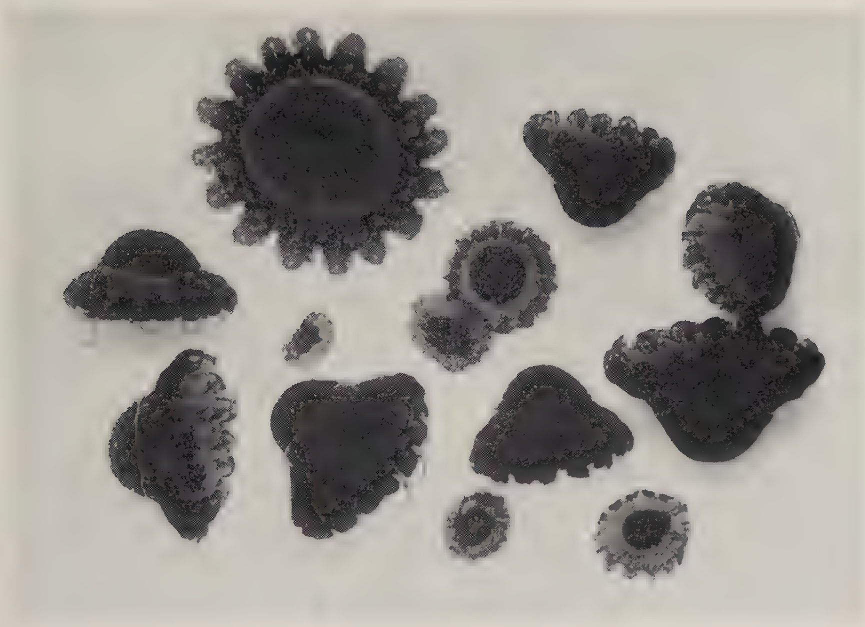

Fig. 1

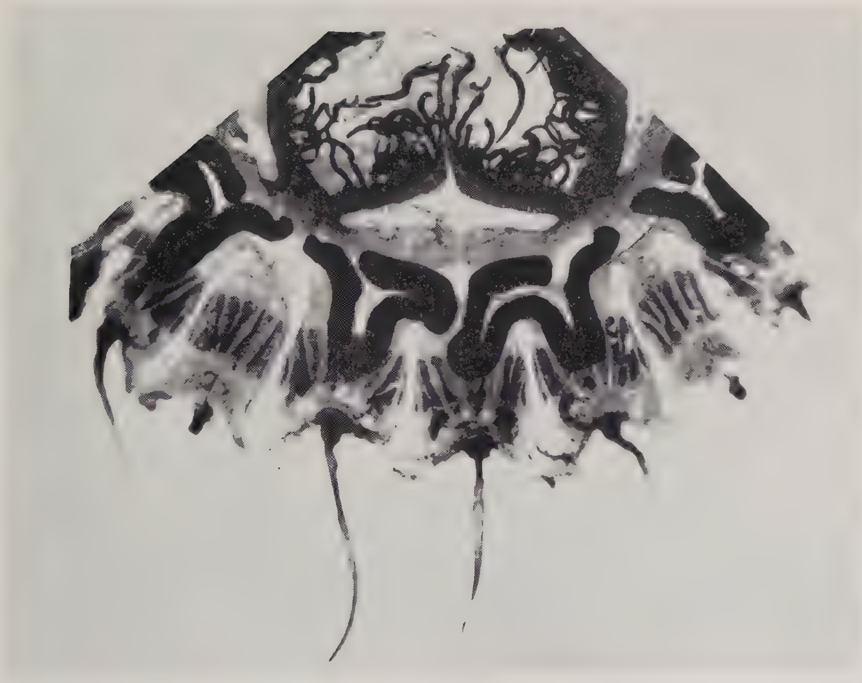

Fig. 2

(*Facing p.* 110)

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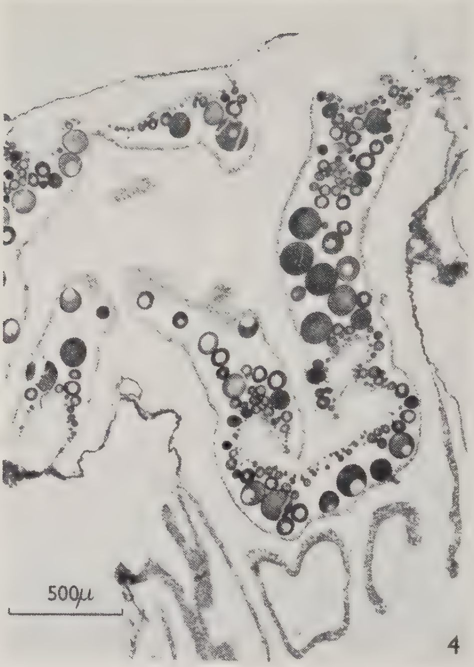

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EXPLANATION OF PLATES

PLATE I. *Paraphyllina ransoni*

Fig. 1. Photograph of specimens about four-fifths natural size; one specimen has been pressed on to the bottom of the glass vessel to show the marginal lappets.

Fig. 2. Sector of umbrella bleached by Mayer's chlorine method, stained with borax carmine, and mounted.

PLATE II. *Paraphyllina ransoni*

Figs. 1, 2. Two halves of the umbrella of the same specimen showing reduction of gonads (male); bleached, stained and mounted as in Pl. I, fig. 2.

Fig. 3. Radial section through rhopalium, stained with haematoxylin and eosin.

Fig. 4. Section through female gonad, stained with haematoxylin and eosin.

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THE HOST-SPECIFICITY, MICRO-ECOLOGY, ADHESIVE ATTITUDES, AND COMPARATIVE MORPHOLOGY OF SOME TREMATODE GILL PARASITES

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(Plates I and II, and Text-fig. 1)

In recent years there have been several accounts of the occurrence of diclidophoroidean trematodes parasitic on the gills of fishes, e.g. Price (1943), Sproston (1946), Dawes (1947), Brinkmann (1952), and Chauhan (1953), and these accounts have included descriptions of the morphology of the parasites. The distribution records have revealed a generally high degree of host specificity and, in some species, a preference even for certain gill arches of the particular hosts, while the morphological descriptions have shown that there is a considerable variation in the form of the parasites, extending to various degrees of deviation from bilateral symmetry (Pl. I, figs. 1–11). These morphological variations are present in spite of the fact that the different parasites occupy such broadly similar habitats in the gill chambers of their respective hosts. But, as far as I am aware, this is the first attempt to investigate the distribution and morphology of the parasites in relation to their micro-habitats.

MATERIAL AND TECHNIQUES

Most of the material used in the present study was collected at Plymouth during the months of July and August in 1953, 1954 and 1955, but specimens of *Discocotyle sagittata* on *Salmo trutta* were obtained from Breconshire, Carmarthenshire, Monmouthshire and Shropshire. Some material collected previously (Rees & Llewellyn, 1941) from Cardigan Bay, the Irish Atlantic Slope, and the Porcupine Bank has also been used.

The parasites and hosts studied are listed in Table I. The names of the fishes are those used in the *Plymouth Marine Fauna*, 3rd ed. (Marine Biological Association, in Press), and the parasites, with the two exceptions noted below, have been named as in Sproston's synopsis of the Monogenea (1946). The name *Kuhnia scombri* Sproston, 1946, has been shown (Dollfus, 1946; Llewellyn, 1956) to be a synonym of *Octostoma scombri* Kuhn, 1829, and *Cyclocotyla chrysophryi* (van Beneden & Hesse, 1864), Price, 1943, has been used in preference to *Choricotyle chrysophryi* van Beneden & Hesse. The parasite referred to in Table I as 'microcotylid species' awaits precise identification: over seventy species of *Microcotyle* have so far been reported (Sproston, 1946), and so the possible addition of a new species becomes a considerable taxonomic task. In the present study it is important only that a microcotylid kind of diclidophoroidean has been found to parasitize a host *Trachurus trachurus* already harbouring *Gastrocotyle trachuri* and *Pseudaxine trachuri*.

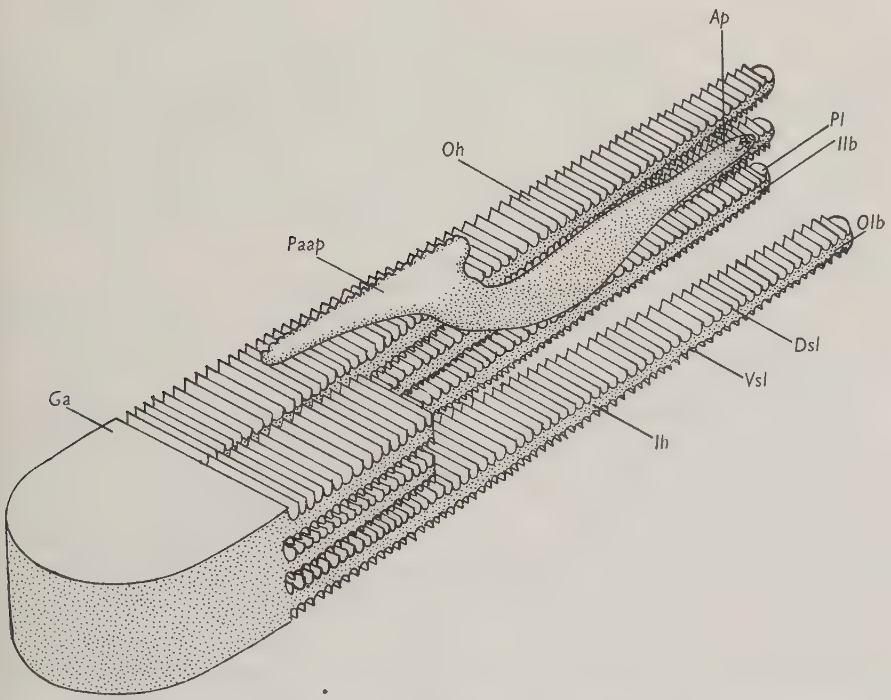
Hosts known from previous records (Baylis & Jones, 1933; Rees & Llewellyn, 1941; Sproston, 1946; Dawes, 1947) to be subject to infection with various diclidophoroideans were examined, as far as was possible, while still alive, the fishes having been brought to the laboratory in baths of sea water. With the parasite of the trout, I have not yet succeeded in examining a living specimen attached to its host: on those occasions when I have taken a microscope to the river bank, my angling friends have not produced a parasitized fish. On other occasions the parasites have died or become detached or have been histologically fixed before examination.

The gills of the fishes were examined by one of two methods, dependent upon previous knowledge of the usual size of the mature parasite. With those fishes, namely *Merluccius merluccius*, *Gadus merlangus* and *G. luscus*, known to be subject to infection with comparatively large parasites, the operculum was lifted and the gills searched while still *in situ*. Sites of infection were then examined carefully with a stereomicroscope. The particular site of infection was noted, i.e. left or right of the fish, serial number of the gill arch, position on the gill arch, inner or outer hemibranch, and dorsal, ventral, or lateral surface of primary lamellae. The importance of a knowledge of all these factors was not realized until the investigation had been in progress for some time, and so early records were incomplete. With hosts likely to harbour smaller parasites, all the gills were removed for microscopic examination. Care was taken so to arrange the excised gill arches that, by inspection, their normal orientation on the fish could be established and so yield records of the precise sites of attachment of the parasites as described above for the larger forms. On only one occasion was a larval trematode observed, but this scarcity could have been due to the loss of larvae after the capture of the hosts, or to insufficiently close observation.

As many as possible of the species of parasites were photographed *in situ* on the gill lamellae, and these have been reproduced in Pl. II. In *Axine belones* the body is bent in two planes, rendering photography very difficult, and a stereogram has been drawn (Text-fig. 1). This figure also illustrates the anatomical terms used for gill structures.

For the preparation of whole mounts for the study of body symmetry, specimens were relaxed either in 7½% magnesium chloride solution, or by using menthol, or by simply allowing them to remain in sea water until they were dead. They were then fixed in either Bouin's or Gilson's fluid without the usual pressure of a cover-glass, stained in haemalum, and mounted in

Canada Balsam. Serial paraffin sections were prepared of some specimens of all the parasite species *in situ* on the gills, the sections being stained by Heidenhain's Azan or Masson's trichrome methods.



Text-fig. 1. Stereogram of *Axine belones* on the gills of *Belone belone*. (Very diagrammatic.) *Ap*, anterior end of parasite; *Dsl*, dorsal secondary lamella; *Ga*, gill arch; *Ih*, inner hemibranch; *Ilb*, inner lateral border of primary lamella; *Oh*, outer hemibranch; *Olb*, outer lateral border of primary lamella; *Paap*, posterior adhesive apparatus of parasite; *Pl*, primary lamella; *Vsl*, ventral secondary lamella.

Host Specificity

In the combined earlier investigations of the Irish Sea, the Irish Atlantic Slope, and the Porcupine Bank (Rees & Llewellyn, 1941) and the present Plymouth investigations, 2104 host specimens belonging to seventeen fish species have been examined, and over 900 parasites belonging to eighteen diclidophoroidean species have been collected. Except for *Plectanocotyle gurnardi*, which I have collected from three different species of *Trigla*, namely *T. cuculus*, *T. lineata* and *T. gurnardus*, and *Diclidophora minor* (usually parasitic on *Gadus poutassou*), of which a single specimen was found on a host which I identified as *Gadus merlangus*, all parasite species have been found to be strictly specific to their particular hosts. Only one host has yielded more than one species of parasite, namely *Trachurus trachurus* often harbouring together both *Gastrocotyle trachuri* and *Pseudaxine trachuri*, and, in addition, on a single occasion one specimen of an unidentified microcotylid species.

DEGREE OF INFESTATION OF SOME HOST SPECIES, AND DISTRIBUTION OF THE PARASITES ON THE GILL ARCHES

The collections at Plymouth in 1953–5 were made primarily for the purpose of obtaining parasitic specimens, and the sizes of the samples of the various host species depended on several contingencies. These included the actual yield of parasites, relatively smaller numbers of the hosts found to be the more highly infected being examined; the method of examination of the host, microscopic examination taking much longer than macroscopic (see p. 114); the relative frequency of occurrence of certain hosts in the Plymouth area; and the availability of certain hosts, for while relatively large numbers of fishes were supplied by the Marine Biological Association's trawlers, smaller numbers were obtained from commercial vessels and from anglers.

In making records of the infestation of fishes by gill trematodes the rudimentary first gill arch was ignored, and the remaining arches were numbered 1 to 4 in anterior-posterior succession. The results are included in Table I.

From an inspection of the results in Table I, the following general conclusions may be drawn. First, it is quite clear that *Diclidophora merlangi* occurs most frequently on the first gill arch of *Gadus merlangus*, *Diclidophora luscae* on the second and third gill arches of *Gadus luscus*, and *Anthocotyle merluccii* on the second gill arch of *Merluccius merluccius*. Secondly, there is a tendency for *Plectanocotyle gurnardi* to occur less frequently on the first gill than upon the other gills of *Trigla cuculus*, and similarly a tendency for *Gastrocotyle trachuri* to occur less frequently on the fourth gill than upon the other gills of *Trachurus trachurus*. Thirdly, with *Axine belones* on *Belone belone*, there is a tendency for the first and fourth gills to be less heavily parasitized than the second and third. Insufficient numbers of the remaining species were available for significant comment.

THE MORPHOLOGY AND ADHESIVE ATTITUDES OF THE PARASITES IN RELATION TO THEIR HOSTS

Without exception the parasites were found to be attached with their posterior adhesive organs nearer to the gill arch of the host, and with the anterior end nearer to the distal end of the primary lamellae. In this way the attached ends of the worms lie upstream relative to the gill ventilating current of the host, with the mouth of the parasite downstream.

Beyond this common general adhesive attitude, the parasites exhibited further similarity in that all but two of the species examined were found to attach themselves to the secondary gill lamellae of their hosts (Pl. II, fig. 1).

The exceptional species were *Anthocotyle merluccii* on *Merluccius merluccius* (Pl. II, figs. 5, 6), and *Cyclocotyla chrysophryi* on *Pagellus centrodontus* (Pl. II, fig. 9), where the principal attachment is more directly to primary lamellae. In those parasites which adhere to the secondary lamellae, the opening of each of the attachment organs is directed obliquely postero-ventrally with respect to the parasite, i.e. the plane between the two valves of each attachment organ lies parallel to those which would normally be occupied by the secondary gill lamellae when washed over by the respiratory current of the host (Pl. II, fig. 1).

TABLE I. DEGREE OF INFESTATION OF HOSTS, AND DISTRIBUTION OF PARASITES ON GILL ARCHES, OF FISHES EXAMINED AT PLYMOUTH 1953–55

tegory ost of mple*	Host	No. of host specimens examined	infected	Infestation (%)	Parasite	Total numbers of parasites per gill arch 1	2	3	4
A	*Gadus merlangus*	507	44	8·7	*Diclidophora merlangi*	53	8	1	4
	G. luscus	509	108	21·0	*D. luscae*	7	118	91	8
	Merluccius merluccius	500	38	7·6	*Anthocotyle merluccii*	4	28	7	5
B	*Trigla cuculus*	20	19	95·0	*Plectanocotyle gurnardi*	6	22	39	25
	Trachurus trachurus	37	23	62·2	*Gastrocotyle trachuri*	41	56	46	20
			8	21·6	*Pseudaxine trachuri*	8	9	5	2
			1	2·7	Microcotylid species	Not known			
C	*Belone belone*	18	14	77·8	*Axine belones*	19	29	48	22
	Scomber scombrus	8	8	100·0	*Octostoma scombri*	7	15	1	0
D	*Pagellus centrodontus*	47	1	2·1	*Cyclocotyla chrysophryi*	0	0	1	0
	Morone labrax	3	2	66·7	*Microcotyle labracis*	3	0	0	0
	Gadus pollachius	15	1	6·7	*Diclidophora pollachii*	Not known			
	Alosa fallax	2	1	50·0	*Mazocraes alosae*	Not known			

A, hosts readily available, searched macroscopically; B, hosts readily available, searched microscopically; , hosts less readily available, searched microscopically; D, hosts less readily available, searched acroscopically.

Beyond the common general features described above, the parasites investigated were found to exhibit considerable variation in their adhesive attitudes. The variable factors include attachment either to one or to more than one primary lamella, attachment to a particular hemibranch, and disposition of the body either between primary lamellae of the same hemibranch or between primary lamellae of different hemibranchs, i.e. between the two hemibranchs of a gill. The pattern of this variation was found to be constant for particular species of parasite and will be described in the following pages, where an attempt is also made to relate the morphology of each of the parasites to its characteristic adhesive attitude.

In *Plectanocotyle gurnardi*, on the gills of *Trigla cuculus* (Pl. II, fig. 2), *Octostoma scombri*, on the gills of *Scomber scombrus*, *Mazocraes alosae*, on the gills of *Alosa fallax*, *Discocotyle sagittata*, on the gills of *Salmo trutta*, and

Microcotyle labracis, on the gills of *Morone labrax*, the posterior adhesive organs are applied either all to the secondary lamellae of the dorsal surface of a primary lamella or all to the secondary lamellae of the ventral surface of a primary lamella, of either an inner or an outer hemibranch, i.e. the trematode is attached to one side or the other of a primary lamella, and never to both sides. In fact the distance between the members of a pair of symmetrical adhesive organs is quite insufficient to span the interval between dorsal and ventral secondary lamellae, i.e. the lateral border, devoid of secondary lamellae, between the dorsal and ventral secondary lamellae.

The longitudinal axis of the parasite, throughout its whole course, always lies parallel to the long axis of the primary lamella, and the whole parasite is bilaterally symmetrical. The attachment organs are all either sessile or borne on very short peduncles. Median ventral posterior hooks (anchors) are present in *Octostoma*, *Mazocraes* and *Plectanocotyle*, but absent in *Discocotyle* and *Microcotyle*.

Further illustrations of the adhesive attitude of the above group of species, as exemplified by *Octostoma scombri*, are given elsewhere (Llewellyn, 1956).

Diclidophora luscae, on the gills of *Gadus luscus* (Pl. II, fig. 3), *D. denticulata*, on the gills of *Gadus virens*, and *D. phycidis*, on the gills of *Urophycis blennoides*, attach themselves to their respective hosts in such a manner that the members of a pair of symmetrical adhesive organs are each applied to opposite sides of the same primary lamella, so that one member of the pair adheres to the secondary lamellae of the dorsal surface and the other to the secondary lamella of the ventral surface. The adhesive organs in these three species of *Diclidophora* are borne on peduncles, the function of which is clearly that of extending the width of the body, and so assisting the parasite to span the distance between the dorsal and ventral surfaces of a primary lamella. As a result of this method of attachment the median longitudinal axis of the worm lies along a lateral border of a primary lamella. The body is bilaterally symmetrical, and no median posterior hooks are present in the adult parasites.

Diclidophora merlangi, on the gills of *Gadus merlangus* (Pl. II, fig. 4), spans both dorsal and ventral surfaces of primary lamellae, as do *Diclidophora denticulata*, *D. luscae*, and *D. phycidis*, but the first-named trematode differs from the other three in that its first and second pairs of adhesive organs are spread over more than one primary lamella, whereas in the other three species all the adhesive organs are applied to the same primary lamella. Characteristically the third and fourth pairs of adhesive organs of *D. merlangi* each grasps the same primary lamella; the second pair grasps the primary lamellae in the same hemibranch immediately on each side of that grasped by the third and fourth pairs; and the first pair of adhesive organs grasps the lamellae that are situated next but one to those grasped by the third and fourth pairs. Occasionally the first pair of adhesive organs may grasp the same lamellae as those grasped by the second pair.

Compatible with this adhesive attitude which involves the spanning of several primary lamellae, the body of *D. merlangi* in the region of the first and second pairs of adhesive organs is relatively wider than the corresponding regions of *D. denticulata* and *D. luscae*, and moreover, the peduncles of the first two pairs of adhesive organs are relatively long. The body is bilaterally symmetrical, and no median posterior hooks are present.

In *Cyclocotyla chrysophryi*, on the gills of *Pagellus centrodontus* (Pl. II, fig. 9), the attachment organs function as suckers and not as clamps (see Llewellyn, 1941), i.e. the adhesive organs do not have opposable jaws for the grasping of pairs of opposite surfaces of primary or secondary lamellae. Instead, each sucker may be applied to any relatively large smooth surface. In the single living specimen that I have had opportunity to examine microscopically, the suckers were applied to the outer lateral borders of primary lamellae, i.e. to regions devoid of secondary lamellae. The four pairs of adhesive organs are borne on relatively long peduncles, and may be spread over two, three, or four adjacent primary lamellae. When forcibly detached from the gill surface, the suckers were able readily to re-establish themselves. When first observed on the living host the parasite was attached to the lateral borders of primary lamellae, but on detachment soon effected new attachment, not only to primary lamellae, but also to the gill arch itself, as shown in Pl. II, fig. 9.

The body is bilaterally symmetrical, and no median posterior hooks are present.

In *Anthocotyle merluccii*, on the gills of *Merluccius merluccius* (Pl. II, figs. 5, 6), the anterior-most of its four pairs of attachment organs is relatively enormously developed so that each of the members of the first pair of organs is itself able to span the distance between the dorsal and ventral surfaces of a primary lamella. The parasite uses this large pair of clamps to grasp the inner border of a primary lamella of an inner hemibranch, the three remaining pairs of small adhesive organs becoming attached to secondary lamellae of the dorsal surface of the same primary lamellae. The body of the parasite passes between primary lamellae of the outer hemibranch and then bends at right angles so that its dorsal surface is in contact with the outer surface of the hemibranch. Were the longitudinal axis of the body to remain in one straight line, the body of the parasite would now lie with its length across the gill ventilating current. However, asymmetrical development has resulted in a bending of the body in the region immediately anterior to the large adhesive organs so that the long axis of the parasite comes to lie more nearly parallel to the direction of the gill ventilating current, and thus the parasite offers less resistance to this current. In a sample of forty-two specimens of *Anthocotyle merluccii* the inclination of the body was found to be towards the animal's right in sixteen (Pl. I, fig. 8, where the animal is seen in ventral view) and to the animal's left in the remaining twenty-six. All twenty-six parasites in which the inclination of the body was to the left came from gills of the left side of the host fish, and all sixteen with right-inclined bodies came from gills of the right side.

A further feature of asymmetry was found in the relatively greater development of the member of the first pair of adhesive organs which grasps the primary lamella at a position relatively distal to the gill arch (Pl. II, fig. 6). This larger clamp may be on the left or right of the parasite, but is always on the downstream side with respect to the gill-ventilating current of the host, and thus always on the same side towards which the asymmetrical body is inclined. In conformity with the apparently exacting and obligatory requirements of the adhesive attitude of *A. merluccii*, it follows that parasites from the left side of the host fish will be inclined to their left and will have the larger clamp on the left side, and similarly with respect to the right.

Median posterior hooks are present.

In *Axine belones*, on the gills of *Belone belone* (Pl. II, figs. 7, 8A, 8B, and Text-fig. 1), the number of adhesive organs is greatly increased from the more usual six or eight to a number varying between about fifty and seventy. Moreover, in *Axine belones* these posterior adhesive organs, instead of being borne in two symmetrical rows, one on each side of the body, are borne in a single oblique row on what is apparently one margin of the body. This margin is not constantly on the same side of the body in all specimens, at least with respect to the marginal vaginal aperture. This latter organ was found, in a sample of twenty-five specimens, to be without exception on the animal's left. In a sample of 100 specimens of *Axine belones*, the posterior adhesive organs were found on the parasite's right in eighty-seven cases, and on the left in the remaining thirteen cases. Hooks that are presumably homologous with the posterior median hooks of *Octostoma*, *Mazocraes* and *Plectanocotyle* are present approximately mid-way along the row of adhesive organs.

The median longitudinal axis of the body of *Axine* is inclined to the line of the row of adhesive organs at an angle of about 30°.

During attachment to the host, the adhesive organs are applied to secondary lamellae of either the dorsal or the ventral surface of a primary lamella, but always near to the *outer* lateral border of a primary lamella of either the inner or outer hemibranchs. The row of adhesive organs lies parallel to the lateral border of the primary lamella so that the body of the parasite, being inclined to the row of adhesive organs, extends beyond the inner lateral border of the lamella to which it is attached. In the region where the body of the parasite crosses this inner border of the lamella, the body bends at right angles to the plane of the posterior adhesive apparatus so that the greater part of the body of the parasite comes to lie between the two hemibranchs of a gill. As a result of this adhesive attitude the longitudinal axis of the greater part of the body of *Axine*, i.e. the part lying between two hemibranchs of a host gill, lies parallel to the gill-ventilating current of *Belone*. The characteristic disposition of the body is illustrated in Pl. II, fig. 8, where in fig. 8A the posterior adhesive region is seen in ventral view, and the remainder of the body in lateral view, and where in fig. 8B the body of the same specimen is seen in ventral view.

In *Pseudaxine trachuri*, on the gills of *Trachurus trachurus*, there are about twenty to thirty posterior adhesive organs, all borne in a single row along one margin only of the body. Ventral hooks are present at the extreme posterior end of the row of clamps. The longitudinal axis of the body is inclined to the row of adhesive organs at an angle varying between about 30 and 50°. These adhesive organs are applied near the outer lateral borders of the relatively narrow primary lamellae, and so the body of the parasite soon crosses the inner border of the primary lamella. Here it bends usually through a right angle so that the greater part of the body of the parasite lies between two hemibranchs as in *Axine* (Pl. II, fig. 7; and Text-fig. 1), but more occasionally through 180°, so that the body of the parasite is in contact with the opposite side of the same primary lamella to that to which its adhesive organs are attached.

All twenty-four specimens of *Pseudaxine* that I have collected were found attached near to the distal ends of primary lamellae.

In a sample of sixteen specimens of *P. trachuri*, the adhesive organs were found on the right of eleven animals, and on the left of the remaining 5.

Gastrocotyle trachuri, on the gills of *Trachurus trachurus* (Pl. II, fig. 10), is asymmetrical, the twenty-five to thirty posterior adhesive organs being confined to one side only of the body. The row of adhesive organs lies approximately parallel to the longitudinal axis of the body of the parasite, and ventral hooks are present at the extreme posterior end of the body. In a sample of seventy-eight specimens of *Gastrocotyle trachuri* the adhesive organs were found on the left of thirty-nine animals, and on the right of the other thirty-nine.

The parasite may be attached to any of the secondary gill lamellae, irrespective of whether they be those of the dorsal or ventral surfaces of a primary lamella, or whether the primary lamella belongs to an inner or an outer hemibranch. But invariably the parasite lies with its attachment organs nearer to the outer border of a primary lamella (i.e. the outer border of a hemibranch) so that a (small) part of the body overlaps the inner border of the same primary lamella and comes to lie between two hemibranchs. In the region where it overlaps into the space between the hemibranchs, the body of the parasite is folded ventrally through 90°.

Discussion

The present investigation of the distribution of diclidophoroidean parasites on Plymouth fishes has shown these trematodes to be entirely specific to their particular hosts. My single previous record of *Diclidophora minor* from *Gadus merlangus* very probably involved mis-identification of the fish, for in an unpublished thesis (1940), in the library of the University of Wales, I referred to this host as '*Gadus merlangus* var. Deep-Sea', probably on account of the trawlermen's name of 'Deep-Sea Whiting' for the fish. Gallien (1937) had previously found *Diclidophora minor* on *Gadus poutassou* in the same locality, and it is almost certain that my specimen of *Diclidophora minor* came from the same host species. It seems distinctly possible also that the record of Baylis & Jones (1933) of *D. denticulata* from *Merluccius merluccius* at Plymouth was also a case of mistaken identity, for the 500 specimens of *M. merluccius* examined in the present study were parasitized only by *Anthocotyle merluccii*. Among the British marine Diclidophoroidea, then, only *Plectanocotyle gurnardi* appears to enjoy the hospitality of more than one host species, and in this case the hosts are closely related. A detailed examination, therefore, should be made of specimens of *Plectanocotyle gurnardi* from *Trigla gurnardus*, *T. lineata*, and *T. cuculus* for signs of incipient speciation.

The descriptions of the adhesive attitudes and of the morphology of the diclidophoroideans studied have shown that there is an exacting topographical relationship between parasite and host, and this is probably an important factor in the mechanism of host specificity.

The present investigation has confirmed Cerfontaine's (1898) observations that the maximum incidence of *Diclidophora merlangi* is on the first gill of *Gadus merlangus*, and that that of *Diclidophora luscae* is upon the second and third gills of *Gadus luscus*. On the other hand, Frankland (1955) found that her observations on a third *Diclidophora* species on a *Gadus* host, namely *Diclidophora denticulata* on *Gadus virens*, gave different results from those of Cerfontaine. The latter author (1898) had found the parasite to be most prevalent on the second and third arches, but Frankland, in a sample of 247 host specimens, found the first and second arches to be the most heavily parasitized. Cerfontaine made no reference to his technique of collecting, and did not record any larval or small forms. Frankland, however, made a microscopic search of the gills, and recorded the presence of larvae. The inference is that there is a change in the maximum incidence of parasite per gill arch with the age of the parasites, the shift, in *Diclidophora denticulata*, being from the first and second arches at initial infestation, to the second and third arches when older. If this is so, it would require either that the parasites are capable of transferring themselves from one gill arch to another, to which there is some contrary evidence (Frankland, 1955), or else that the degree of survival of parasites initially attaching themselves to the second and third gills is greater than for those attaching themselves to the first. In future studies it might be possible to test the latter hypothesis by investigating if there is any correlation between the degree of development of the parasite (size?) and the particular site of infestation.

Cerfontaine thought the explanation of the differential distribution among the gill arches to be in a choice of gill exercised by the parasite at initial infestation, but in the absence of any evidence that the infective parasite is able to swim well enough to manoeuvre itself in the gill ventilating current, it is difficult to see how any such selective power could operate.

An alternative explanation would be to assume that the infective larvae are swept involuntarily over the gills by the gill-ventilating current. Any variations in the volume of water passing over the different gill arches might then be reflected in the numbers of opportunities for parasites to become attached. However, the greater numbers of larvae brought to those gills receiving the greater ventilation would themselves be committed to a lifelong struggle against the greater current, and the survival rate would be correspondingly lower than on less well-ventilated gills. Those parasites that change their adhesive attitudes during their lifetime, e.g. *D. denticulata* changing from the larval manner of attachment on one side of the primary lamella (Frankland, 1955) to the adult manner of attachment on both sides, appear to be particularly vulnerable at the time of effecting this transfer.

As to the difference in prevalence per gill arch between, on the one hand *D. luscae* and *D. denticulata*, each on the second and third gills of their respective hosts, and on the other hand *D. merlangi*, on the first gill of its host, the most profitable approach for understanding the problem appears to lie in a detailed comparison of the gill ventilating mechanisms of *Gadus luscus*, *G. virens*, and *G. merlangus*.

A common feature of the adhesive attitude found in all the parasites here studied is that the posterior adhesive organs are attached to the gills at a position upstream relative to the gill ventilating current, with the anterior mouth-bearing end downstream. Such an adhesive attitude is obviously well adapted to meet what appear to be the major ecological problems facing these parasites, namely those of clinging to the host in the face of a current, and of feeding, from the highly vascular secondary lamellae, on the blood that forms the main part of their diet (Llewellyn, 1954).

The particular forms of adhesive attitude have been found to vary considerably. The simplest method, e.g. *Octostoma*, *Mazocraes*, *Plectanocotyle*, *Discocotyle* and *Microcotyle*, is for the parasite to apply the whole of its body to the secondary lamellae of one surface of a primary lamella. It thus lies completely between primary lamellae of one hemibranch, and no part of the body is exposed to what presumably are the stronger currents between and outside the hemibranchs.

A variation occurs in *Diclidophora* species. In *D. luscae*, *D. denticulata* and *D. phycidis*, the adhesive organs are applied to both the dorsal and the ventral secondary lamellae of the same primary lamella, and in *D. merlangi* the parasite spans several primary lamellae. This adhesive attitude of *Diclidophora*, however, is achieved at the cost of losing suitable anchorage, in the form of secondary lamellae, for median posterior hooks, which are not found in this genus. The adhesive attitude in *Diclidophora* necessitates a widening of the span of the pairs of adhesive organs by means of an increase in body width, and in *D. merlangi*, by the development of relatively long peduncles as well.

Cyclocotyla chrysophryi resembles *Diclidophora merlangi* in that it spans several primary lamellae, but differs from it in that instead of using *clamps* to attach itself to secondary lamellae, it uses *suckers* to attach itself to the smooth lateral borders of primary lamellae. The possession of these suckers permits the parasite to wander over other smooth surfaces, and I have observed *Cyclocotyla* to attach itself firmly to the gill arch of a (dead) *Pagellus*. However, the parasite was observed to contain abundant haematin, which has been shown (Llewellyn, 1954) to be evidence of a blood-feeding habit, and it seems probable that the normal habitat of the parasite on the living host is on the gills. Similarly, the numerous previous records of cyclocotylid parasites from the mouth-cavities of their hosts, or even as super-parasites of crustaceans which are themselves parasitic in the mouth-cavity of the host fish (see Sproston, 1946), are probably the result of the wanderings of which these parasites are capable in virtue of their possession of sucker-like adhesive organs and not clamps.

Anthocotyle merluccii is asymmetrical, and the advantage of the asymmetry is obvious since it results in the long axis of the body coming to lie parallel to the gill-ventilating current instead of across it. The need for this asymmetrical adjustment appears to have arisen from the change in the manner of attachment: the adhesive attitude of the ancestral diclidophoroidean probably resembled that of the present-day *Octostoma*, but the adoption of a habit of attachment by means of enlarged anterior clamps applied to the lateral border of a primary lamella necessitated a shift in the orientation of the parasite to a position at right-angles to the gill-ventilating current. A natural consequence would be the development of asymmetry to regain a body disposition offering less resistance to the water current. The reason for the unequal development of the members of the anterior-most pair of clamps is less clear. One would expect the adhesive organ farther upstream to bear the greater burden and so to be the more powerful and presumably the larger. In fact, however, the reverse obtained without exception in a sample of forty-two specimens of *Anthocotyle* observed *in situ* on the gills of *Merluccius*: the clamp nearer to the gill arch was invariably the smaller one of the pair. It has been shown (p. 120) that the side of the parasite on which the larger clamp occurs is directly related to the side of the fish upon which the parasite occurs. Since this appears to be purely a matter of chance, the side of the occurrence of the larger clamp has no taxonomic significance, and Brinkmann's (1952) relegation to synonymy with *Anthocotyle merluccii* of an American species erected on grounds of the side of development of the larger clamp was fully justified.

The asymmetry of *Axine belones*, *Pseudaxine trachuri* and *Gastrocotyle trachuri* fulfils the same function as that in *Anthocotyle*, namely that of bringing the longitudinal axis of the body to lie parallel to the gill-ventilating current, but the reason why its development should have been necessary is not clear. Presumably it is directly related to the relative disposition of the long axis of the body and the hinge axes of the clamps during early development. This, however, itself presents certain problems, for in a preliminary examination of the orientation of the clamps of *Axine belones*, it was found that the clamps anterior to the hooks, and also those posterior to the hooks, all face in the same direction. The two sets of clamps are thus not mirror images of one another, as would be expected if their disposition were the result simply of the suppression in growth of the longitudinal axis of the posterior adhesive region of the body as has been suggested by Sproston (1946). More complex factors are obviously involved, and await further investigation.

The direction of asymmetry in *Axine belones*, *Pseudaxine trachuri* and *Gastrocotyle trachuri* has been shown to be dependent upon the particular site of infection of the host. In *Gastrocotyle* this is probably a matter of chance (thirty-nine with clamps on the parasite's left, and thirty-nine on the right, in a sample of seventy-eight), but in *Axine* the unequal distribution (eighty-seven with clamps on the parasite's right, and thirteen on the left, in a sample of a hundred) suggests the presence of a dominating factor influencing the position of the parasite along the curved gill arch, or some other variation. On the other hand, Sproston (1946, pp. 453–4) stated that in a sample of at least thirty specimens of *Axine belones* on *Belone belone* at Plymouth, all but one had the adhesive organs on the *left*. It must be pointed out, however, that Sproston (1946, figs. 104*a*, *b*) illustrated a specimen of *Axine belones* in which the vagina was on the animal's right, whereas I found it, without exception, to be on the left in a sample of twenty-five specimens. Lorenz (1878), in a paper on the anatomy of *Axine*, had also found the vagina on the left. Thus further work will be necessary before the precise topographical relationships between *Axine belones* and *Belone belone* can be determined.

Superficially, the three asymmetrical 'microcotylid' species *Axine belones*, *Pseudaxine trachuri* and *Gastrocotyle trachuri* appear to present a series of progressive reduction, in that the adhesive organs which in *Axine* are posterior to the ventral hooks are lost in *Pseudaxine* and *Gastrocotyle*, and that the body itself becomes relatively much smaller in *Gastrocotyle*. However, the morphology of *Axine* itself presents certain problems which should be investigated before worth-while speculations may be made upon evolutionary trends.

I am greatly indebted to the Director and Staff of the Plymouth Laboratory for their valuable assistance. I am also grateful to Dr H. M. T. Frankland for allowing me to read her manuscript on the bionomics and life history of *Diclidophora denticulata* before its publication.

SUMMARY

In a sample of over 2000 fishes belonging to seventeen species, the gills were found to be infested with over 900 parasites belonging to eighteen species of diclidophoroidean trematodes. All the parasites were found to be specific to particular hosts, with the exception of *Plectanocotyle gurnardi*, which may parasitize any one of three species of *Trigla*.

In most cases there was a characteristic differential distribution of the parasite among the gill arches of the host, and it is suggested that this is the result of variations in the flow of water over the different gills rather than of a choice exercised by the parasite.

The parasites were all attached with the posterior adhesive organs upstream relative to the gill-ventilating current and the mouth downstream. Variations in the adhesive attitude, characteristic of species or groups of species, occurred within this common pattern.

Variations in the form of the parasites such as the width of the body, degree of development of the peduncles of the adhesive organs, and asymmetry, could in each case be related to the characteristic adhesive attitude of the species.

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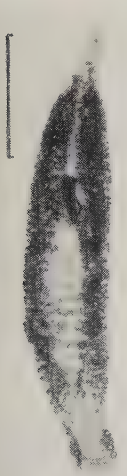

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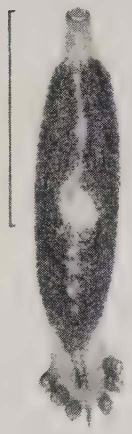

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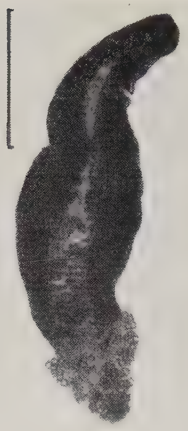

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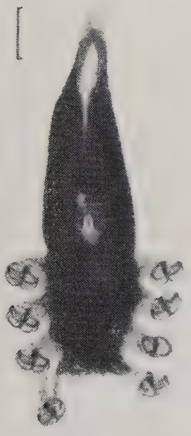

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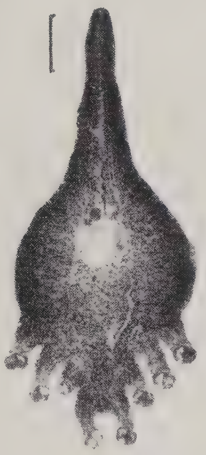

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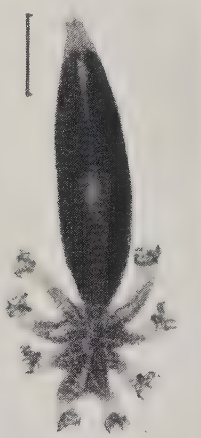

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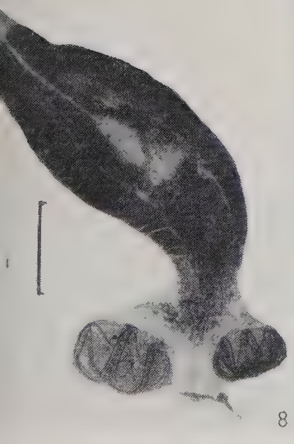

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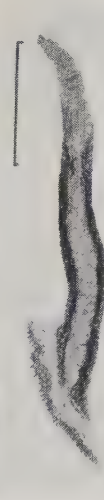

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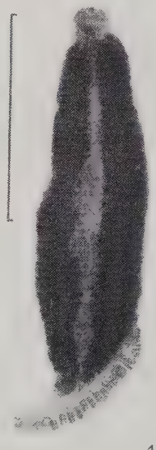

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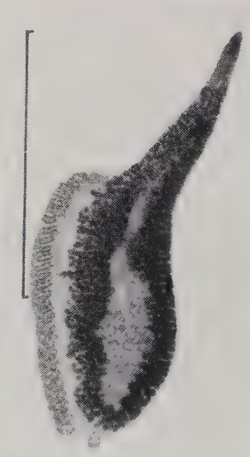

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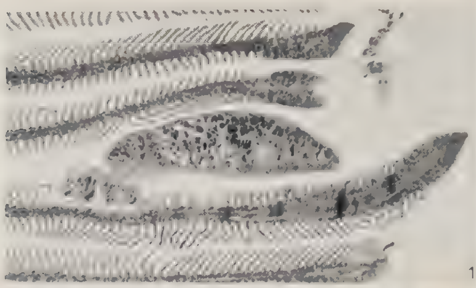
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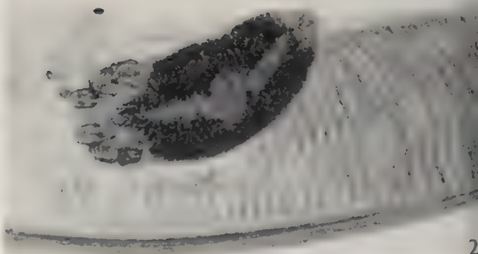
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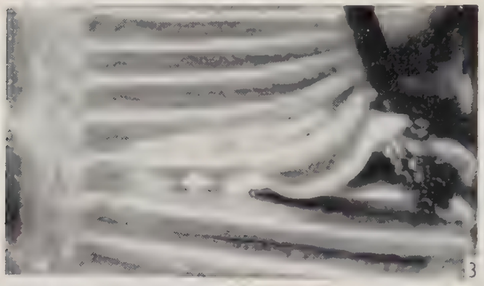
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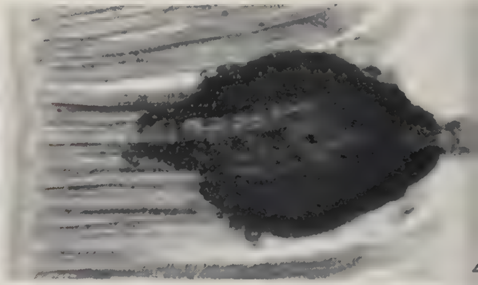

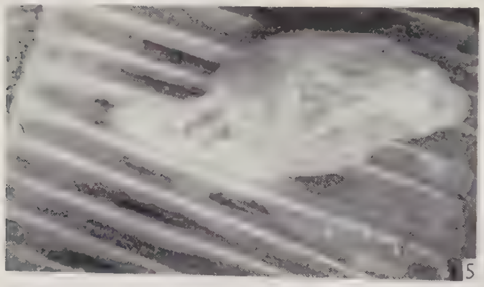
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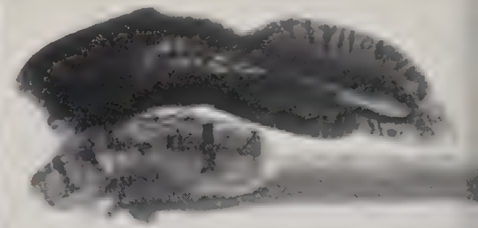
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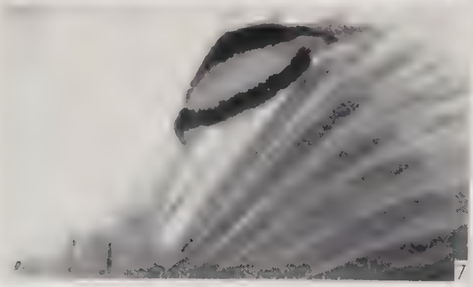
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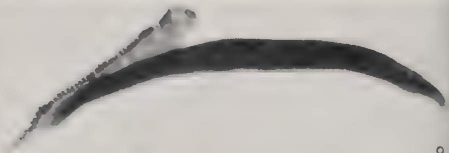
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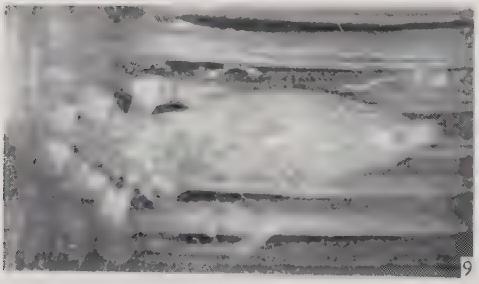
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Direction of gill-ventilating current

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EXPLANATION OF PLATES

PLATE I

The range of form exhibited by representative diclidophoroidean gill trematodes. (Specimens flattened under pressure of cover-glass and mounted in Canada Balsam. Index-line in all figures = 1·0 mm.)

Fig. 1. *Octostoma scombri.*
Fig. 2. *Plectanocotyle gurnardi.*
Fig. 3. *Discocotyle sagittata.*
Fig. 4. *Microcotyle* sp. (from *Serranus cabrilla*).
Fig. 5. *Diclidophora denticulata.*
Fig. 6. *D. merlangi.*
Fig. 7. *Cyclocotyla chrysophryi.*
Fig. 8. *Anthocotyle merluccii.*
Fig. 9. *Axine belones.*
Fig. 10. *Pseudaxine trachuri.*
Fig. 11. *Gastrocotyle trachuri.*

PLATE II

Adhesive attitudes of representative diclidophoroidean gill trematodes. (Figs. 3, 5, 7 and 9: living specimens; Figs. 8A, B: specimens fixed, but not flattened; Figs. 1, 2, 4, 6 and 10: specimens in Canada Balsam.)

Fig. 1. L.S. *Discocotyle sagittata* on *Salmo trutta.*
Fig. 2. *Plectanocotyle gurnardi* on *Trigla cuculus.*
Fig. 3. *Diclidophora luscae* on *Gadus luscus.*
Fig. 4. *D. merlangi* on *G. merlangus.*
Fig. 5. *Anthocotyle merluccii* on *Merluccius merluccius.*
Fig. 6. *A. merluccii* on *Merluccius merluccius.*
Fig. 7. *Axine belones* on *Belone belone.*
Fig. 8A. *A. belones,* body in side view.
Fig. 8B. *A. belones,* body in ventral view (same specimen as 8A).
Fig. 9. *Cyclocotyla chrysophryi* on *Pagellus centrodontus.*
Fig. 10. *Gastrocotyle trachuri* on *Trachurus trachurus.*

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RECEPTOR ELEMENTS IN THE MUSCLES OF *LEANDER SERRATUS*

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(Plates I and II, and Text-figs. 1–4)

In continuation of the studies on receptor elements in the Crustacea the common prawn, *Leander serratus*, has been investigated by the methylene-blue method applied in the same way as described in an earlier publication (Alexandrowicz, 1951). It has been found that there are in *Leander* receptors of the same kind as in those crustaceans previously investigated, some of which exhibit features not yet observed before. As in other crustaceans they are similarly situated on the dorsal side of the body, but since a knowledge of the disposition of the dorsal muscles in the thorax and the abdomen is necessary for locating them, some data about the topography of the muscles are first given.

TOPOGRAPHY OF THE DORSAL MUSCLES

The disposition of the muscles as they come to view when exposed from the dorsal side by removal of the carapace and terga is shown in Text-fig. 1. On the left side a part only of the carapace has been removed, and the muscles are left *in situ*. On the right the muscle flanking the heart, which is the first head of the lateral thoracico-abdominal muscle, has been partly removed and the epimeral plate turned with its median surface uppermost. In this way the muscles inserting into this plate become exposed, and on such preparations the receptor elements of the thorax can be examined. It must, however, be borne in mind that in their normal position the muscles follow the curvature of the body and the epimeral plate lies in the dorso-ventral plane.

On pulling aside the posterior part of the first head of the lateral thoracico-abdominal muscle one can see its second head—a flat muscle spreading fanwise with its bundles inserting into the epidermal plate.

In the second large mass of muscles passing from the thorax to the dorsal side of the abdomen three units, called the 1st, 2nd and 3rd dorsal thoracico-abdominal muscles, can be distinguished. All three have their anterior attachments on the epimeral plate. The first dorsal thoracico-abdominal muscle inserts posteriorly into the anterior edge of the first abdominal segment. The second consists of two portions which run side by side anteriorly but separate posteriorly. One portion passes over into the abdomen and fuses end to end with the supplementary extensor muscle of the first abdominal segment; the second portion passes under the intermediate plate and helps to form a bulky muscle filling up the concavity of this plate (Text-figs. 1, 2).[1] The 3rd dorsal thoracico-abdominal muscle, the stoutest of all, consists of three portions with separate attachments on the epimeral plate. They pass posteriorly under the intermediate plate, but a part of the stoutest portion is interrupted by a constriction at the anterior edge of this plate. The arrangement of the muscles situated under the intermediate plate is complicated, since it is here that the fibres of various parts of the thoracico-abdominal muscles and also of the extensor muscles of the abdomen meet. The anterior border of the latter is marked by a tendinous intersection which can be seen when the overlying fibres of the thoracic muscles are pulled aside (Text-fig. 2A).

The terms designating the muscles are the same as those used in the work on *Homarus* and *Palinurus* (Alexandrowicz, 1952*a*) and follow, with some minor additions, the nomenclature of Schmidt (1915). It should be emphasized that although there is similarity in the general plan of the musculature of the Macrura there are also differences, and it cannot be claimed that all the muscle units to which the same name has been applied correspond in fact to each other. Besides, the above description is a simplified one and many details in the drawings had to be omitted.

In the abdomen the muscles whose situation should be known when considering the receptor organs are the extensors. Two sets of these muscles which will be called the main and the supplementary extensors can be distinguished. The main extensors, which make up nearly all the mass of this musculature, have their bundles arranged in various ways. Those situated nearer the median line of the body are twisted around each other, while those in the lateral portion take a straighter course. The relations of the bundles of these portions, their courses and modes of attachment are very complicated and cannot be entered upon here.

The term 'supplementary extensors' is proposed for designation of superficially situated flat muscles with parallel or slightly diverging fibres which are never twisted. Posteriorly, in all five segments, they are attached to the anterior edge of the next segment, but they exhibit differences in their length and anterior insertions. In the first segment this muscle is ribbon-shaped and runs forwards and outwards to continue, as aforesaid, with the thoracico-abdominal muscle. In the second segment its shape and direction are the same, but it ends near the anterior border of the segment. In the following

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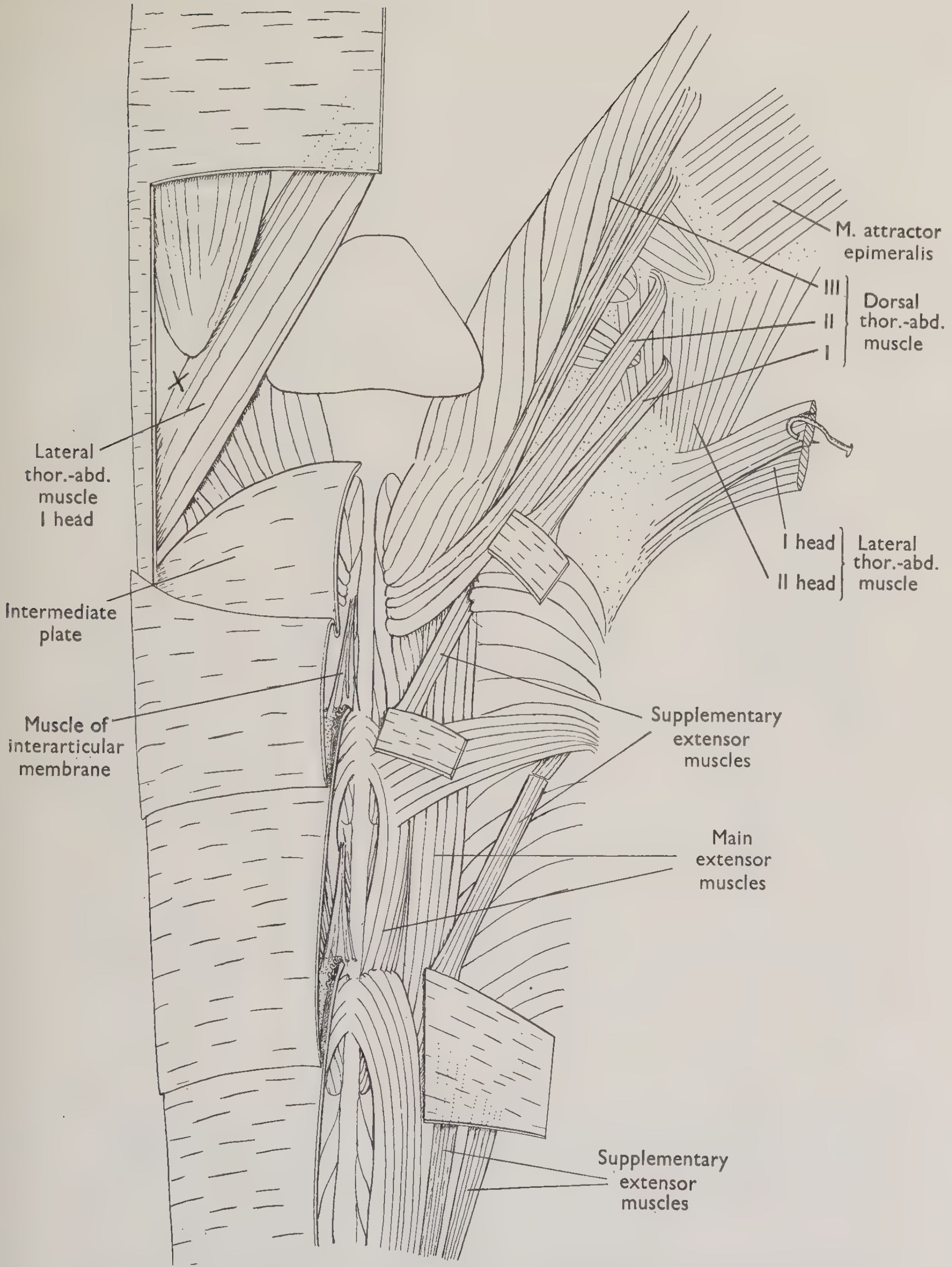
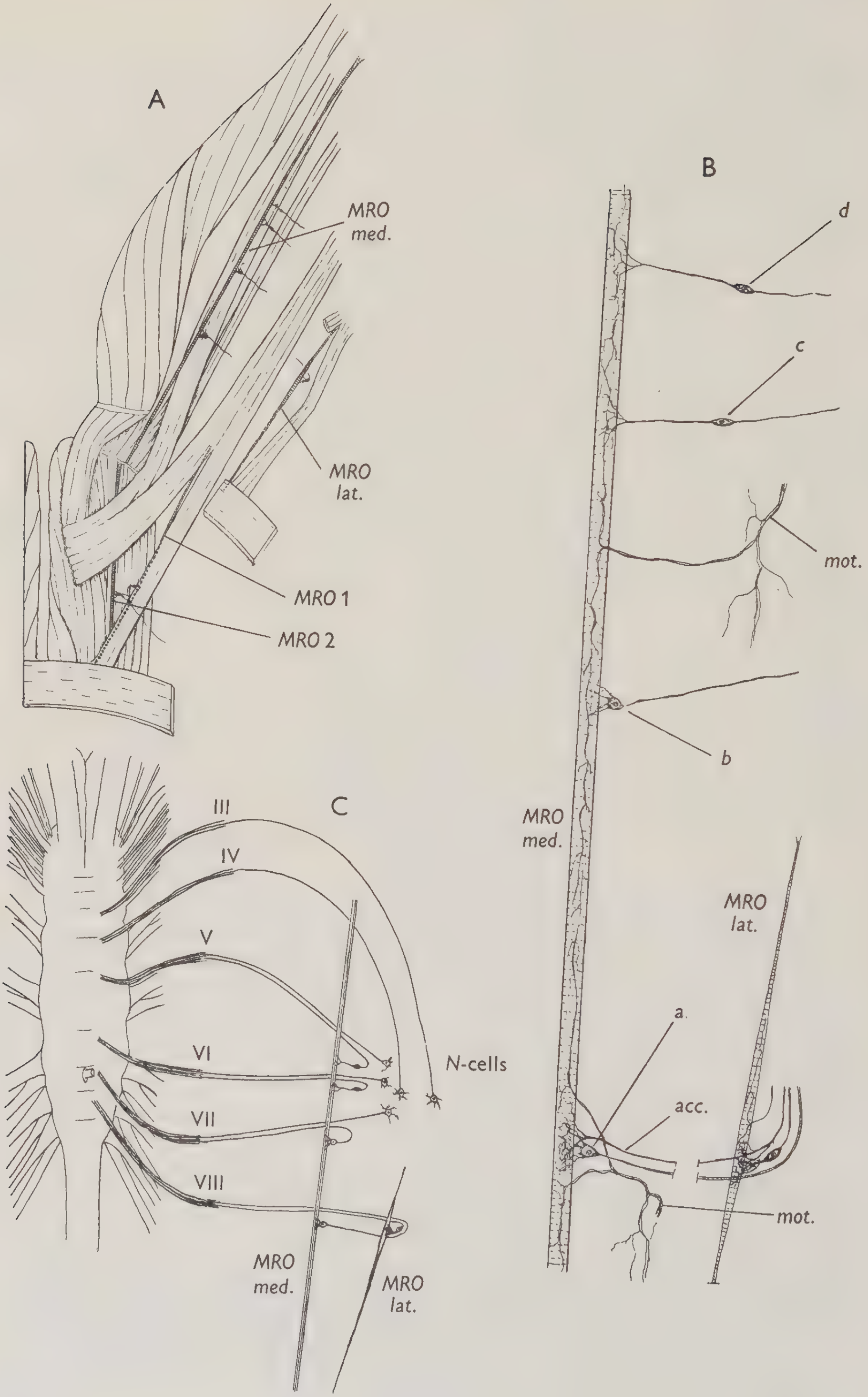
[1] The text-books, even the most comprehensive, skip over this region, and although its chitinous covering, as in *Leander*, forms a conspicuous part of the skeleton it appears not to have a generally adopted name. The term 'intermediate plate' has been used by Calman (1904). Patwardhan (1937), in his monograph of *Palaemon*, calls it in the explanation of his fig. 1 'arthrodial membrane between the thorax and the first segment of the abdomen' and in the text says: 'The posterior half of the inter-tergal membrane connecting the cephalothorax with the tergum is calcified and rigid....' I am grateful to Dr Marie V. Lebour for pointing out to me these two sources of information.


M. attractor epimeralis
III
II
I
Dorsal thor.-abd. muscle
Lateral thor.-abd. muscle I head
I head
II head
Lateral thor.-abd. muscle
Intermediate plate
Muscle of interarticular membrane
Supplementary extensor muscles
Main extensor muscles
Supplementary extensor muscles

Text-fig. 1. *Leander serratus*. Topography of the dorsal muscles in the thorax and three abdominal segments seen from the dorsal side. On the left, the carapace has been partly sectioned. On the right, the carapace and the terga are removed except for small parts of the latter with the insertions of the supplementary muscles; the first head of the lateral thoracico-abdominal muscle is sectioned and its posterior portion hooked outwards; the epimeral plate is turned with its median surface upwards. The cross on the left side marks the position of an *N*-cell.


A
MRO med.
MRO lat.
MRO 1
MRO 2
B
d
c
mot.
b
MRO med.
MRO lat.
a.
acc.
mot.
C
III
IV
V
VI
VII
VIII
N-cells
MRO med.
MRO lat.

Text-fig. 2.

segments the muscles become gradually shorter, wider, and divide into two or three portions with slightly diverging fibres. They insert into the tergum at a distance from its anterior border which is gradually greater in the subsequent segments (Text-fig. 3). It is to be noted that the histological structure of various units of the dorsal musculature is not the same, viz. the supplementary abdominal extensors and the 1st and 2nd thoracico-abdominal muscles have a more coarse cross-striation.

The term supplementary muscles, used here for facilitating the description, can be misleading as it may imply a subordinate role. It is true that these muscles are weak, but considering their different structure there is a ground for assuming that they must have a special function. Moreover, as similar differences have been observed in other crustaceans (Macrura, Stomatopoda, Amphipoda), it can be inferred that these animals have two systems of the extensor muscles presumably one for slow and the other for fast contractions. It is also for this reason that a special term seems to be appropriate.

Receptor Elements

Two categories of receptors in the muscles of *Leander* can be distinguished.

(1) Muscle receptor organs (*MRO*) consisting of nerve cells connected with special receptor muscles (*RM*). As in other crustaceans previously investigated—(*Homarus vulgaris*, *Palinurus vulgaris*, *Pagurus striatus*, *P. calidus*, *Squilla mantis* (Alexandrowicz, 1951, 1952*a*, *b*, 1954), *Cambarus clarkii* (Wiersma, Furshpan & Florey 1953), they are of two sorts (*MRO* 1, *MRO* 2).

(2) Receptor cells, called *N*-cells, which end in ordinary muscles and have been found, as in *Homarus* and *Palinurus*, in certain thoracic muscles.

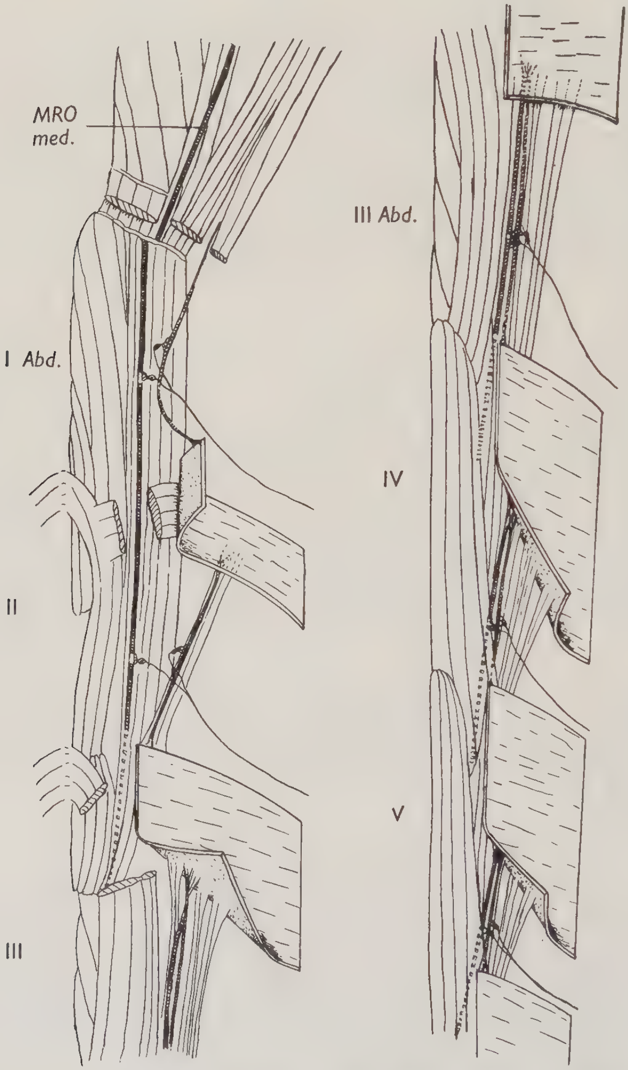
Muscle Receptor Organs in the Thorax

Two muscle receptor organs have been found in the thorax, and according to their situation they can be called the lateral and the median *MRO*, the same terms as used in the work on *Homarus* and *Palinurus*.

Legend to Text-fig. 2

Text-fig. 2. *Leander serratus.* A, Topography of the muscle receptor organs in the thorax and in the first abdominal segment. The dorsal thoracico-abdominal muscles are spread apart (cf. Text-fig. 1); the 1st dorsal thoracico-abdominal muscle is partly removed. *MRO lat.*, *med.*, lateral and median muscle receptor organ of the thorax; *MRO* 1, *MRO* 2, muscle receptor organs of the 1st abdominal segment. B, Muscle receptor organs of the thorax (semi-diagrammatic). Only the middle part of the median *MRO* and the anterior part of the lateral *MRO* are represented (cf. C). Note the situation of the nerve cells *a* and *b* close to the muscle and of the cells *c* and *d* at a distance from it. *mot.*, motor fibres; *acc.*, accessory nerve. C, Diagram showing the connexions of the thoracic receptor cells with the suboesophageal ganglion. III–VIII, nerve trunks of the 3rd–8th segment carrying axons of the receptor cells of the *MRO* and of the *N*-cells.

The lateral *MRO* lies between the bundles of the 1st dorsal thoracico-abdominal muscle (Text-fig. 2A). Its muscle component has in its middle part the shape of a spindle with its thickest part (*c.* 35μ) lying nearer to the anterior end. This spindle thins out in both directions to a fine thread, only *c.* 10μ thick. Both its attachments take place near to those of the 1st dorsal



Text-fig. 3. *Leander serratus.* Topography of the muscle receptor organs in five (I–V) abdominal segments. Note the varying length and shape of the supplementary extensor muscles (removed in the first segment), the gradual shortening of the receptor muscles in the 3rd–5th segment, and the fusion of the muscles of the *MRO*2 in the 1st and 2nd segments. Parts of receptor muscles covered by muscle fibres are drawn in dotted lines.

thoracico-abdominal muscle, but the receptor muscle, although it is so thin and runs between the other muscle fibres, can be traced as an individual unit.

The nerve cell of this *MRO* is bipolar with dendritic processes starting with a common root (Pl. I, fig. 2). They expand in the thicker part of the muscle in dense arborizations (not seen in the photograph). The axon joins the nerve trunk belonging to the 8th thoracic segment. The situation of this receptor is very unfavourable, both for staining and observation. To see its muscle it is necessary to pull aside the muscle fibres covering it; the nerve cell is hidden from view by the nerve fibres curving round the thoracico-abdominal muscle. Moreover, the same nerve trunk conveys some deviating fibres of the pericardial organs which at this point break up in fine branches arranged as small glomeruli. To see the cell as shown in the photograph all these elements have to be removed, but the operation is very delicate.

It is difficult to get a clear picture of the nerves supplying this receptor. There is certainly a motor fibre for the muscle and there are also fibres of the accessory innervation intermingling with the arborizations of the cell-dendrites, but whether they derive from one or two accessory nerves I am unable to say.

The median thoracic *MRO* can be found much more easily. In preparations with the median side of the epimeral plate exposed as in Text-fig. 1 it can be seen on the surface of the middle portion of the 3rd dorsal thoracico-abdominal muscle (Text-fig. 2A). Anteriorly it ends near the insertions of the adjacent muscle; running backwards it passes deeper under the bundles of the 3rd dorsal thoracico-abdominal muscle, and its posterior insertion into the connective tissue septum between the thoracic and abdominal extensors can be seen only after the superficial layer of the muscles in the region of the intermediate plate is pulled aside. The muscle of the median *MRO* is much thicker than that of the lateral one, measuring 50–60μ in diameter. Nearing the anterior end it becomes thinner. There are a good many connective tissue fibres running longitudinally on the periphery of the muscle and between the bundles of the myofibrils. Towards the anterior end the connective fibres gradually predominate over the muscle elements.

Nerve Cells

Four nerve cells are connected with the median receptor muscle. Their positions as shown in Text-fig. 2B and C are the most frequent, but variations in the situation of the three anterior cells are common. In one preparation all three have been found lying close together (Pl. I, fig. 4).

There are certain differences in the appearance of the cells. The posterior one (Text-fig. 2B*a*; Pl. I, fig. 1) is multipolar; it is situated close to the receptor muscle, sending into it dendrites spreading over an area the length of which is equal to approximately three diameters of the cell. The tuft of arborizations is well delimited on both sides. In this region the myofibrils of the muscle, at least most of them, are replaced by connective tissue fibres.

The next cell *b* is usually multipolar and very similar to cell *a*, but often shows variations in its shape; sometimes with all the dendrites arising with a common root it looks like the anterior cells *c* and *d*. The latter are bipolar with distal processes which are of such a length that the cells as a rule lie much farther from the muscle than the cells *a* and *b* (Pl. I, fig. 3). Here again variations are not uncommon, and one or the other cell can have a multipolar shape and lie close on the muscle.

The dendrites of the cell *b* show the pattern of arborizations similar to those of the cell *a*, but are often more irregularly delimited. The dendrites of the two anterior cells are less densely arranged, but can be much longer than in cell *a*. The expansions of the three cells presumably end on the connective fibres, but the picture is not clear as the myofibril bundles pass through these areas.

The variable appearance of the terminations of the three anterior cells may be partly due to the unevenness of the staining, but evidently also because they are affected by frequent irregularities. As we see later this feature can help us in understanding the nature of these elements.

The axons of the four cells are directed at first outwards and, after joining the nerve trunks running on the median side of the epimeral plate, turn ventrally to proceed towards the suboesophageal ganglion: the axon of the posterior cell on curving round the thoracico-abdominal muscle is joined by the axon of the lateral *MRO*, and both travel in the nerve trunk of the 8th thoracic segment. The three other axons belong most probably to the 5th-7th segments, as shown in Text-fig. 2C. Some uncertainties arise when tracing these axons on account of the anastomoses occurring between the nerve trunks of various segments in which they travel. Moreover, the axons of the cells *b* and *c* or *c* and *d* are sometimes to be seen running side by side; it can be assumed that such associations are accidental and that these fibres in their farther course part company.

The nerves carrying the axons of the receptor organs belong to the set of nerve trunks originating in the suboesophageal ganglion on its dorsal side. They include the motor nerves of the thoracic muscles, the various nerves of the heart, of the pericardial organs and also the axons of the *N*-cells (see below).

Nerves

The motor fibres for the median receptor muscle branch from the nerves of the adjacent muscles. One fibre approaches the receptor muscle near the posterior cell and another in front of it in the region between the two middle cells (Text-fig. 2B). Each of them gives off branches running in the muscle in both directions.

Only the posterior cell has an accessory innervation limited to one fibre, which can be qualified as the thick accessory nerve (Pl. I, fig. 1). The thin accessory nerve seems to be missing.

Muscle Receptor Organs of the Abdomen

In the abdomen, muscle receptor organs have been found in the 1st to 5th segments. In the 6th they are most probably missing, but as the particular arrangement of the muscles in this segment makes the dissection difficult there is no absolute certainty as to this point. In the first two segments the two receptor units of one side (*MRO* 1 and *MRO* 2) are separated from each other, whereas in the 3rd-5th segments they lie close together.

The muscle receptor of the *MRO* 1 of the first segment (*RM* 1) has its forward insertion in the region of the intermediate plate between the bundles of the 2nd dorsal thoracico-abdominal muscle (Text-fig. 2A). From this point it runs obliquely backwards and inwards close to the supplementary extensor passing on its ventral side near its inner edge. Its posterior attachment is on the anterior border of the tergum of the 2nd segment. This *RM* 1, like the lateral thoracic *RM*, has a spindle-shaped middle part continuing in both directions with thin threads. The diameter of this thin part at the mid-point between its anterior attachment and the nerve cell was found to be 8 μ.

The muscle of the second more medially situated receptor *RM* 2 inserts anteriorly quite near the posterior end of the median thoracic *MRO* into the septum separating the thoracic and abdominal extensors (Text-fig. 3). In its backwards course, which is almost parallel to the median line of the body, it lies on the lateral portion of the main extensor muscle. At the border between the 1st segment and the 2nd segment it fuses end to end with the *RM* 2 of the 2nd segment, so that actually there is one receptor muscle running through the first two segments and passing into the anterior part of the third segment where it ends among the bundles of the main extensor muscle. Its total length in middle-sized specimens is about 2 cm, and the diameter which does not change much at various levels is about 50 μ. The position of this *RM* is superficial in these regions, where the muscle bundles on which it is lying are not covered by other muscles. In order to see it in its whole length these overlying muscles must be removed (Text-fig. 3). It is somewhat difficult to isolate the receptor muscle in the region between the two segments, and it is possible that the connective tissue fibres accompanying the *RM* 2 have here some points of attachment, but the continuity of the muscle can be ascertained.

The *RM* 1 of the second segment resembles that of the first segment in its dimensions and direction of its course. It lies on the supplementary muscle near its median margin, and its insertions are near to those of this muscle (Text-fig. 3).

In the 3rd-5th segments the two *MRO* units are situated near to each other, and the anterior attachments of both *RM* are at the same level (Pl. II, fig. 7). Posteriorly each *RM* 1 inserts into the anterior edge of the tergum of the following segment, whereas the *RM* 2 runs farther backwards and passing deeper into the layer of the main extensor muscle ends between the bundles of the latter. The length of both *RM* decreases from the 3rd to 5th segment and so does the calibre of the *RM* 2. The *RM* 1 retains about the same thickness in its middle part and does not taper so much as in the first segments. Already in the 3rd segment the differences between the two *RM* are not so great as in the first two segments, and in the 5th segment the two muscles are almost of the same calibre. In the diagrammatic drawings (Text-fig. 3) the diameters of the *RM* are out of scale. Their true proportions can best be understood from the photographs (Pl. II, figs. 7, 8) made from the same preparation of the *MRO* of the 3rd segment. The represented parts of the muscles added together include only about half of their total length.

In all segments the receptor muscles are accompanied by connective tissue fibres running longitudinally; even in the thinnest parts of the *RM* 1 the elements of both the muscle and connective tissue are present. Connective tissue also replaces all or almost all the myofibrils of *RM* 2 in the area of expansion of the cell dendrites. In *RM* 1 a good deal of myofibrils can be seen passing over this area. Some difference in cross-striation of the muscles as recorded previously in the large Macrura and Stomatopoda can be observed, viz. the *RM* 1 has a coarser and *RM* 2 a finer cross-striation.

Nerve cells

The nerve cells in the abdominal *MRO* are situated laterally to the muscles, with exception of the cells of the *MRO* 1 in the 1st and 2nd segments, in which they lie either directly above the muscles or medially to them. The particular features of the cells of one pair of *MRO* can be better seen in the posterior segments where both receptors lie close to each other. Here they exhibit great resemblance to the pictures observed in *Homarus* (Pl. I, fig. 5). Cell 1 (of the *MRO* 1), which is always situated in front of cell 2, has wider extending processes than the latter in which these processes and all their ramifications are closer to each other. Consequently, the areas of the respective muscles in which the arborizations of the cells are interlaced with the end-branches of the accessory nerves differ markedly in size and shape (Pl. I, fig. 6).

In Pl. I, fig. 5, representing the receptors of the 5th segment, cell 1 is larger of the two. In the 1st–4th segments the cells are about the same size.

The cells are surrounded by membranaceous connective tissue. There is possibly a cavity separating the cells from this capsule as in large Macrura and Stomatopoda, but it is not distinct enough. Fine nerve fibrils wind round cell 2 forming a so-called basket-work. Similar fine fibres, but not so numerous, can sometimes be noticed on the periphery of cell 1.

Nerves

The pattern of the motor innervation is the usual one, i.e. some of the motor fibres run down alongside the axons of the cells and others branching from the motor nerves of the adjacent muscles reach the *RM* at points lying farther from the cells.

There are many other fibres approaching the cells and entering into relations with their dendrites (Pl. I, fig. 6; Pl. II, fig. 9). Most of them can be traced to their origin from one fibre of stouter calibre—the thick accessory nerve which is conveyed by the same trunk running on the surface of the dorsal muscles into which pass the axons of the receptor cells. In this trunk run also finer fibres which can be regarded as thin accessory nerves.

The thick accessory nerve gives off branches entering into relations with the dendrites of both receptor cells; whether it also supplies both receptor muscles or only the *RM* 1 is not clear. As to the thin accessory nerve it can be assumed that its branches reach the terminations of both cells, but their distribution is not clear. In general, the methylene-blue preparations with *Leander* tissues give less distinct pictures than with the large Macrura. It is therefore not to be expected that such points as appeared dubious in the larger species could be elucidated with this less suitable material.

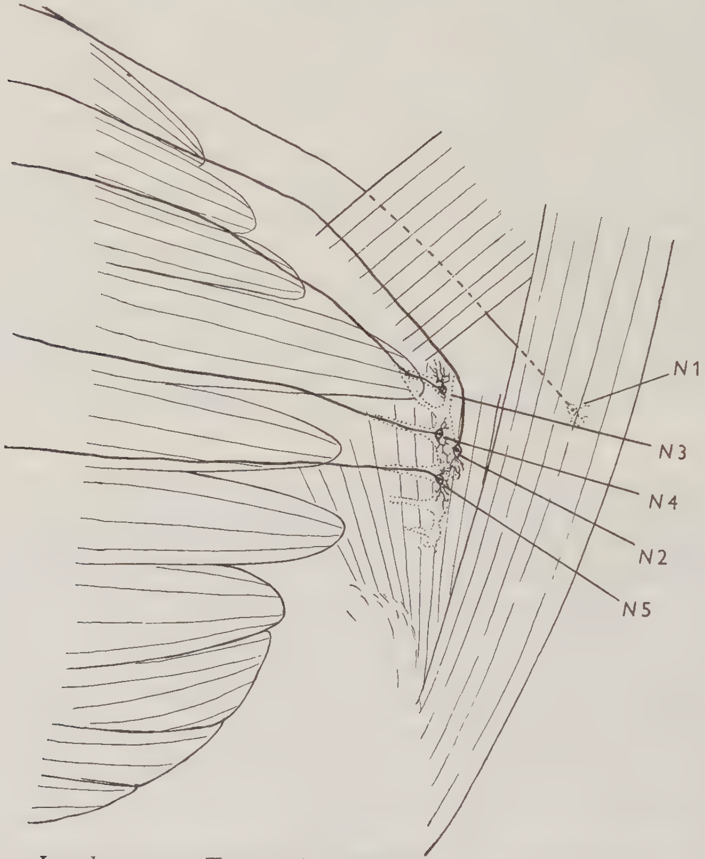
Receptor cells in ordinary muscles (N-cells)

The conventional term *N*-cells has been adopted for designation of nerve cells first described in *Homarus* and *Palinurus*, which send their dendritic expansions into ordinary muscles. In *Leander serratus* five of them have been found. One is situated on the outside of the first head of the lateral thoracico-abdominal muscle; it can be seen in a living animal injected with methylene blue at the point indicated by *X* in Text-fig. 1 on the left side, provided the cuticle is transparent enough. Four other cells lie on the middle bundles of the 2nd head of the lateral thoracico-abdominal muscle, and can be observed only on preparations with the median surface of the epimeral plate turned upwards (Text-fig. 4). The observation is somewhat impaired, as these *N*-cells are situated between the arch-like trunks of the pericardial organs and the muscles and therefore are covered by the former. As there is no connexion between the pericardial organs and the nerve cells it is possible to remove the overlying parts of them without injuring the cells and their processes.

The *N*-cells are about the same size as the nerve cells of the *MRO* or even larger than some of them. They are therefore comparatively larger than in *Homarus* and *Palinurus*, in which their size is always smaller than in *MRO*. The dendrites arising from different points of the cell body penetrate between the bundles of the muscles over a larger area than in the *MRO*. The muscles in these regions do not show any particular changes. The photographs

(Pl. II, figs. 10, 11) give an idea of the general appearance and various forms of the *N*-cells, but cannot show the processes running deeper into the muscles which are out of focus.

Whether the endings of these processes are on the muscle tissue or on connective fibres is difficult to discern, especially as the muscles in crustaceans are not composed of such units as the muscle fibre of vertebrate histology, but consist of bundles of myofibrils anastomosing with one another. Accordingly the connective tissue separating the muscle mass into fasciculi and enveloping the myofibril bundles does not form such regular tubular sheaths as the endomysium of the vertebrate muscle.



Text-fig. 4. *Leander serratus.* Topography of the *N*-cells on the lateral thoracico-abdominal muscle viewed from the inside (cf. Text-fig. 1). Cell *N* 1 situated on the outside of the first head of the muscle is seen by transparency. The proximal course of the axons of the *N*-cells is shown in Text-fig. 2c.

In the description of *N*-cells in *Homarus* more probability has been given to the assumption that their processes end on connective fibres. A corroborative evidence is now afforded by the fact that the dendrites of the most anterior cell *N*3 (Text-fig. 4) can be seen branching on a strip of tissue continuing with the muscle but in which no myofibrils can be noticed.

The axons of the *N*-cells in their course towards the suboesophageal ganglion join each a nervous trunk of a different segment (Text-fig. 2c). The axon of the cell *N*1 situated on the outer side of the lateral thoracico-abdominal muscle enters a branch of the nerve of the 3rd segment, passing on the lateral side of the m. attractor epimeralis. The axons of the four remaining cells run in the trunks of the 4th-7th segments. The cell *N*2 belonging to the 4th segment is situated behind the cell *N*3 of the 5th segment. Its axon crossing m. attractor epimeralis on its inner surface near to its ventral attachment can be easily found in every preparation, as it stains readily even while its cell is pale or invisible.

The axons of the cells *N*3–5 run in the trunks of the 5th–7th segments, which are surrounded by the neuropiles of the pericardial organs. As stated in the paper describing these structures (Alexandrowicz, 1953) no relation between the *N*-cells and the pericardial organs has been found.

Discussion

It will be noted from the above description that the structure and arrangement of receptor elements in the muscles of *Leander*, although similar to that of the large Macrura, have certain peculiar features. One of them is the extraordinary thinness to which one receptor muscle, the *RM*1, can be reduced. In the 1st abdominal segment the ratio of its diameter in the anterior part ($8\,\mu$) to the total length of the muscle would be approximately 1:1000. It is, further, interesting that although both receptor muscles vary in their dimensions in different segments these variations are by far greater in *RM*1. Hence it may be concluded that this muscle needs finer adjustments to the mechanism of movements in each of the segments, presumably conditioned by the differences in the arrangement of their extensor muscles.

The receptors of the second set, the *MRO*2, show two unusual features: the fusion of the muscles of the 1st and 2nd abdominal segments and the occurrence of four cells connecting with one muscle in the thorax. The fusion of the receptor muscles has not been observed, either in the Decapoda or in the Stomatopoda. In some other crustaceans, however, on which some investigations are being made, a fusion of the muscles has been found. Thus in the thorax of *Ligia oceanica* (Isopoda) one receptor muscle runs through two segments, but the nerve cells are in one of them only; in *Praunus flexuosus* (Mysidacea) the muscles of both *MRO* pass through three thoracic segments, and in each of them two nerve cells are present. The functional meaning of such fusions of the muscles is difficult to conceive.

The median receptor organ in the thorax, which obviously belongs to the same set as *MRO* 2 in the abdomen, appears as an anomaly not only because four cells connect with one muscle, but also because one of them has the accessory innervation which the others have not. However, anomalies in the anatomy of the *MRO* in the thorax can be easily explained as resulting from the retrogression of these organs in the segments which have become fused and have lost their motility in the course of the phylogenetic evolution of the Crustacea. As pointed out previously (Alexandrowicz, 1954, p. 105), during this process some *MRO* can disappear and in others certain components can be missing. According to this conception only the last thoracic segment has in *Leander* retained both muscle receptor organs, but in all probability they possess only one of the two accessory nerves. The three anterior cells of the median *MRO* can be regarded as the remnants of receptor organs of the 5th–7th thoracic segments, probably their *MRO* 2. They send their dendrites into the common muscle, that is perhaps made up of fused muscles of several segments, but have lost all their accessory innervation. The irregularities in the shape and position of these cells corroborate the assumption of their being elements of retrograding organs.

The receptor elements of the second category, the *N*-cells, have been found in *Leander* in the same number, five, as in *Homarus* and *Palinurus*. This does not necessarily mean that they are all exactly the same set of homologous elements, because there is no certainty that in each of these animals all *N*-cells have been found. Besides, the arrangement of the cells and the muscles is different, and the homology of the latter has yet to be established.

Examination of the *N*-cells in *Leander* has not helped towards understanding their function. Some features, such as the concentration of four cells on one small strip of muscle, make their role even more enigmatic. In a previous paper (Alexandrowicz, 1952*a*), when surveying the different types of receptor elements, it has been suggested that the *N*-cells might represent a more primitive type from which those with special muscles have developed. However, it is equally conceivable that, when highly specialized muscle receptors are becoming redundant, the reversed process may occur in the course of which their own muscles disappear and the nerve cells enter into relation with the ordinary muscles. In that case the *N*-cells might be the remnants of the retrograding receptor organs, presumably the *MRO* 1. The connexions of their axons with the 3rd–7th thoracic segments would fit into this picture. On the other hand, however, there is nothing in their appearance which would indicate that they are decadent elements. The cells are comparatively large, shapely, and their processes are well developed. In trying to solve the problem of the *N*-cells it would be of much help to know whether they are only in the thorax or whether they occur in other parts of the body also. The present investigation, in which thoracic and abdominal muscles of *c*. 150 specimens were examined, makes the first alternative more probable, but the possibility that some nerve cells have not been detected is still to be reckoned with.

SUMMARY

In *Leander serratus* muscle receptor organs have been found in the thorax and in the 1st–5th segments of the abdomen. In the abdomen they are represented, as in other crustaceans, by two units on each side consisting of a thin muscle connected with nerve cell and supplied by motor and accessory nerves. The two muscles (*RM*1 and *RM*2) of each pair of receptor organs keep separated in the first two abdominal segments, whereas in the 3rd–5th they run close together. The muscles *RM*2 of the 1st and 2nd segments are fused end to end, forming one muscle unit running through the segments and connected in each of them with the receptor cell.

In the thorax two muscle receptor organs, a lateral and a median, are present. The former has the usual components, i.e. one muscle and one nerve cell; the median consists of one muscle and four nerve cells. The axons of the posterior cell of the median receptor and that of the lateral receptor run towards the suboesophageal ganglion in a nerve trunk of the 8th thoracic segment; those of the three anterior cells join the trunks of the 5th–7th segments. It is suggested that these three cells are the remnants of muscle receptor organs which have been reduced following the fusion of the thoracic segments and loss of their motility.

The receptor elements of the second category, the *N*-cells, with the dendrites ending in ordinary muscles, have been found, five in number, in connexion with the lateral thoracico-abdominal muscle. Their axons run into the suboesophageal ganglion with the nerve trunks of the 3rd-7th thoracic segments.

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EXPLANATION OF PLATES I AND II

All photomicrographs were made from preparations of *Leander serratus* stained with methylene blue, fixed in ammonium molybdate and mounted in xylol-dammar.

Plate I. The scale at the bottom applies to all figures.

Fig. 1. Part of the median thoracic muscle receptor organ with its posterior nerve cell. *mot*, motor fibre; *acc*, accessory nerve.

Fig. 2. Middle part of the lateral thoracic receptor organ. *RC*, receptor cell, *RM*, receptor muscle. The outlines of the dendritic arborizations are blurred by a diffuse staining.

Fig. 3. Three anterior cells (*b*, *c*, *d*, cf. Text-fig. 2B) of the right median thoracic *MRO*.

Fig. 4. Three anterior nerve cells of the median thoracic *MRO* bunched together and showing irregularly arranged processes.

Fig. 5. Muscle receptor organs of the 5th abdominal segment (left side). The nerve cell of the *MRO* 1 is larger and is situated anteriorly.

Fig. 6. Muscle receptor organs of the 5th abdominal segment (right side). Note the differences in size and shape of the networks formed by the cell dendrites and the accessory nerves. The nerve cells are faintly stained and are invisible in the photograph.

Plate II. The scale at the bottom applies to figs. 9–11.

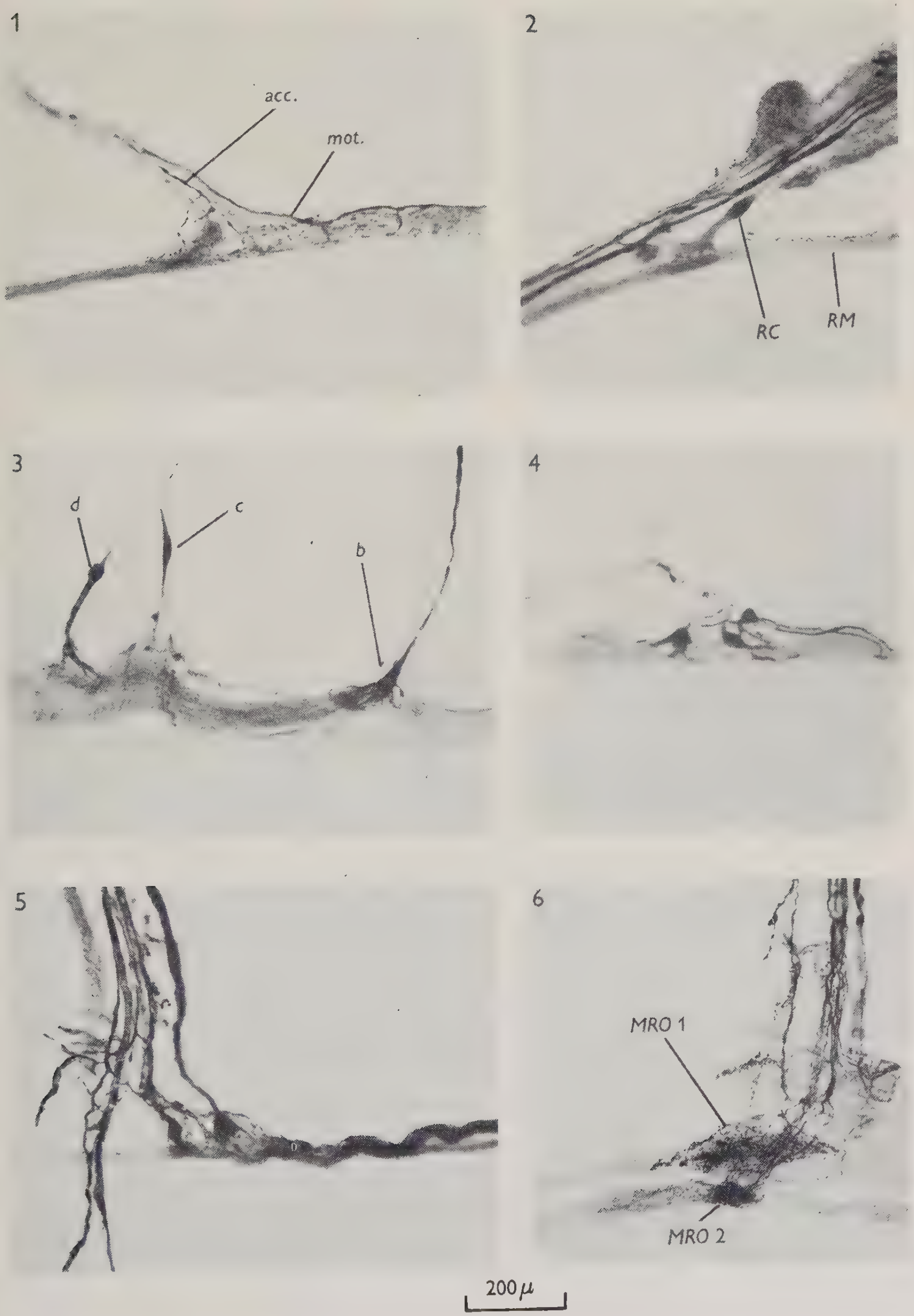
Fig. 7. Middle quarter of the *MRO* of the 3rd abdominal segment of the left side. The receptor muscles became displaced during the mounting of the preparation: in the animal body the *RM* 1 lies dorsal and to the side of the *RM* 2; it passes on its median side in the anterior quarter as seen in fig. 8.

Fig. 8. Same preparation as in fig. 7. Anterior quarter of the *MRO* of the 3rd segment with the attachment of the receptor muscles.

Fig. 9. Motor and accessory nerves of the *MRO* of the 3rd segment. The kink in the muscle and its thickenings are artifacts.

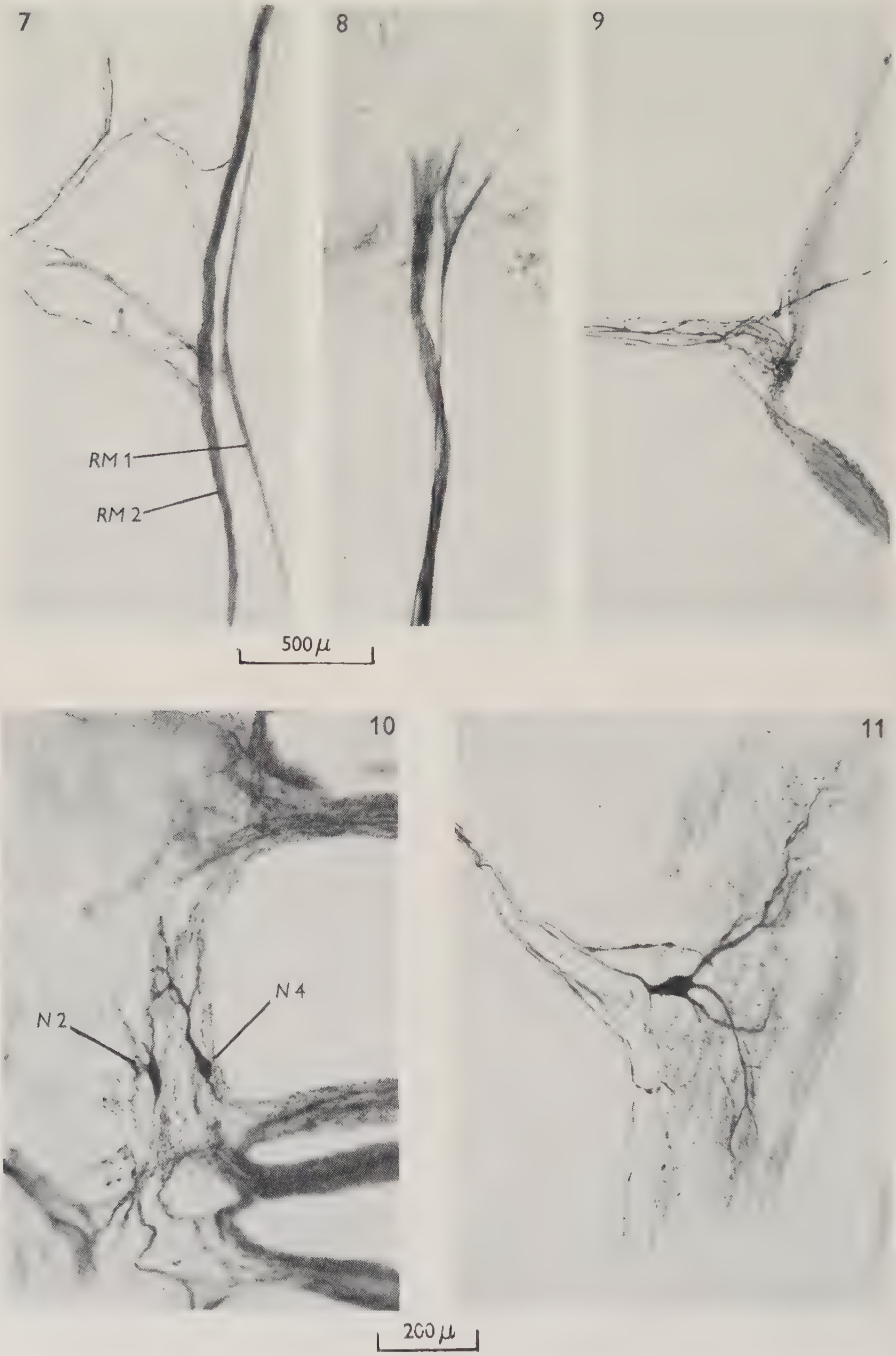
Fig. 10. Two *N*-cells (*N* 2 and *N* 4, Text-fig. 4). The overlying arch-like parts of the pericardial organs are almost completely removed.

Fig. 11. *N*-cell situated on the outside of the first head of the lateral thoracico-abdominal muscle (*N* 1, Text-fig. 4).


1
acc.
mot.
2
RC
RM
3
d
c
b
4
5
6
MRO 1
MRO 2
200 μ

Figs. 1–6.

(Facing p. 144)


7
8
9
RM 1
RM 2
500 μ
10
11
N 2
N 4
200 μ

Figs. 7–11.

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STUDIES ON THE BIOLOGY OF LIMPETS

I. THE LATE J. H. ORTON'S WORK ON *PATELLA*

By A. J. Southward and J. M. Dodd

From the Plymouth Laboratory and the Gatty Marine Laboratory, St Andrews

At the time of his death in 1953 Orton had almost completed an extensive study of the biology of the three British species of *Patella*. Although certain aspects of the investigation were dealt with by collaborators and have already been published (Crewe, 1947 on parasites; Goodwin, 1950 on pigments; Jones, 1948 on ecology) most of the breeding and spawning data have been neither analysed nor published. With the knowledge that Orton's records contained the most complete information on breeding yet compiled for a littoral animal, and that these records had considerable bearing on the question of speciation in *Patella*, we have attempted to analyse and present those which are most complete.

Orton first became interested in the biology of limpets during a general study of growth rate in marine animals in which it became necessary to determine the breeding periods of the animals involved (Orton, 1914, 1920*a*). In *Patella* the preponderance of males in the smaller size-groups and of females in the larger size-groups suggested a change of sex during the life-history similar to that found in other gastropods (Orton, 1909). A preliminary account of the sex proportions (Orton, 1920*b*) was followed by a fuller account in 1928, when the results of investigations on the rate of growth and on shell dimensions were also presented (Orton, 1928*a*, *b*). Other papers dealt with ecology and showed the need for further special studies (Orton, 1929, 1932, 1933).

Orton was unable to resume his investigation of these problems until 1945, and a year later he announced some preliminary results. These confirmed the previous evidence on sex change, and demonstrated differences in the breeding periods of the three British species (Orton, 1946). The investigations proceeded rapidly from this date until his death, the aspects under study including size, sex proportions, breeding cycle, spawning stimulus, rate of growth, parasitization, and general ecology (Orton, 1948, 1949).

To prepare for publication some of the great mass of information collected by Orton, a good deal of selection has had to be practised. In the first place, aspects such as rate of growth, on which the data were very incomplete, have been omitted. The breeding records have been reduced considerably by omitting unnecessary duplicate samples, and small samples. The series of records from Cullercoats has been rejected because of discrepancies between successive samples; in any case this particular limpet population has been studied in detail by other workers (Das & Seshappa, 1947). Records of parasitized neuter individuals have been omitted since the neuter gonad in these cases may be the result of parasitic castration rather than a stage in the normal gonad cycle.

In his later work on the breeding cycle, Orton classified the gonads into ten stages of advancing ripeness and a similar number of post-spawning stages in each sex. These data have been reclassified on the basis of an earlier and simpler scheme which facilitates comparison with other species.

A representative series of gonad stages of *Patella vulgata* was sent to one of us (J. M. D.) by Orton, and these have been used to describe the histology of the gonad. For the macroscopic description of the gonad stages we were fortunate in having available some of Orton's MS. notes and drawings: these also indicated the method which he intended to use for the diagrammatic presentation of the breeding data. The other tables and diagrams are our own, and for these and for the interpretation of the evidence we are wholly responsible.

The nomenclature adopted is based on personal communications from Mr R. Winckworth to Orton, and is the same as that of Brian & Owen (1952). The three British limpets are therefore *P. vulgata* Linnaeus; *P. aspera* Lamarck, 1819 (=*P. athletica* Bean, 1844,=*P. depressa* Jeffreys, 1865); *P. depressa* Pennant, 1777 (=*P. intermedia* Jeffreys, 1865).

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STUDIES ON THE BIOLOGY OF LIMPETS

II. THE BREEDING OF *PATELLA VULGATA* L. IN BRITAIN

By the late J. H. Orton, F.R.S., A. J. Southward
and J. M. Dodd

From the Plymouth Laboratory and the Gatty Marine Laboratory,
St Andrews

(Plates I and II and Text-figs. 1–4)

Some preliminary observations in North Cornwall (Orton, 1946) showed that the periods of breeding of the three British species of limpets were different. In order to determine these differences more accurately, and to study the breeding cycle, samples of limpets from six localities in Britain were examined at regular intervals for 5 years. This paper gives the results obtained for the common limpet *Patella vulgata*, which was present at all the localities sampled and was the most intensively studied of the three species. Later papers will deal with the remaining species and with other aspects of the investigations.

The work fell roughly into two stages. For the first season, 1945–46, it was necessary to determine exactly the distribution of sexes in the various size-groups at the different stations, since it was known that at Plymouth the small sizes were preponderantly male (Orton, 1920, 1928) and roughly equal numbers of both sexes are desirable for regular observations on breeding. At the same time the opportunity was taken to re-investigate sex-phenomena which had been noted previously and thought to indicate a change of sex during the life-history (Orton, 1920, 1928). For the remaining period of study emphasis was placed on determining the exact period of spawning in the various localities, and on possible spawning stimuli.

Although J. H. O. collected most of the samples that were taken personally, as distinct from those collected by other professional or amateur collectors, all three authors have taken part in the field work. J. H. O. was mainly responsible for the examination of the gonad stages, their grouping, and the calculation of the various numerical factors, J. M. D. carried out the histological work and described the arbitrary stages, whilst A. J. S. analysed the breeding cycle and explored the relationship between meteorological factors and spawning. This paper has been prepared by A. J. S. and J. M. D., who are responsible for all opinions expressed herein.

Sampling and Examination of Samples

The regular samples were obtained from the Bay of Nigg, Aberdeen; Pier rocks, St Andrews; Millport, Isle of Cumbrae; Kallow Point, Port St Mary, Isle of Man; Trevone, North Cornwall; and the south side of the breakwater at Plymouth. The collections were usually made at about M.T.L.: the Millport samples, however, were generally taken nearer to H.W.N., while the specimens from Plymouth breakwater were from a ledge half-way between M.T.L. and L.W.N. (see Southward & Orton, 1954, fig. 2, sections C–C and D–D). Additional samples were obtained from other localities in the Isle of Man and near Plymouth during the spawning period to investigate the influence of local factors on spawning.

While many samples were collected personally and examined the same day, a large proportion were sent by post in damp sea-weed, and there was sometimes a delay of up to 4 days between collection and examination of the gonads. The limpets do not seem to have suffered from this treatment: as Eslick (1940) found, they survive well out of water.

The length of the shell was used as an approximate index of size and age. There are no known secondary sexual characters in *Patella*, and the gonad was therefore examined directly, and its stage of development determined, by cutting away the foot from most of its attachment to the visceral mass and shell, and pushing it over anteriorly, thus disclosing the gonad lying beneath the visceral mass. The sexes could usually be separated clearly by colour (Orton, 1928), the male gonad being pinkish white or cream, and the female green or brown (Pl. I), but doubtful cases were examined microscopically. There was no way of distinguishing the sexes of those individuals in the resting phase between spawning and development of the gonad except, in some cases, by microscopic section, and these have been classed simply as the neuter resting stage.

The remaining gonad stages could usually be distinguished as to sex by macroscopic examination. They have been grouped into a series of arbitrary stages, of both development and depletion (spawning), each stage being assigned a roman numeral (Table I). This scheme is based on one put forward in 1910 by a subcommittee of the International Council, for an investigation of herring (cf. Orton, 1916). It differs from the original scheme in distinguishing between recently spent individuals and those in a resting phase prior to recovery of the gonad. Moreover, in the present scheme all stages represent definite increments in the size of the gonad, and it is therefore possible, in a single index, to define the mean gonad condition of the population.

The samples were first grouped into the arbitrary stages by the use of the criteria given below; the numbers of the sexes in each group, and those neuter, were then recorded. From the data so obtained four variables have been extracted by means of which it is possible to define the breeding state of the population sample. One of these is the mean gonad condition, noted above; and the other three are percentages denoting the proportion of the sample in the resting, developing, and spawning condition. The mean gonad condition, or the mean stage of development of the gonad, is determined as follows: the sexes are considered together, since their stage of development is usually identical; the numbers of individuals in each developing gonad stage are multiplied by the number allotted to the stage, the values so obtained being added together. The mean condition of the gonad can then be ascertained by dividing the grand total by the number of individuals in the whole sample.

TABLE I. ARBITRARY SCHEME OF CLASSIFICATION OF GONAD STAGES USED IN THE PRESENT WORK

Stage	Brief description of gonad
Developing	
* o (or N)	Inactive or neuter; either rudimentary virgin stage, or resting after discharge of gametes
I	Beginning to develop, and sex detectable, but only slight increase in size
II	Developing to one-third full size
III	Between one-third and two-thirds full size
IV	Two-thirds full size
V	Fully developed
Spawning	
V+	Full, but actively discharging gametes
IV	Discharging, two-thirds full
III	Discharging, two-thirds to one-third full
II	Discharging, one-third full
I	Almost completely discharged

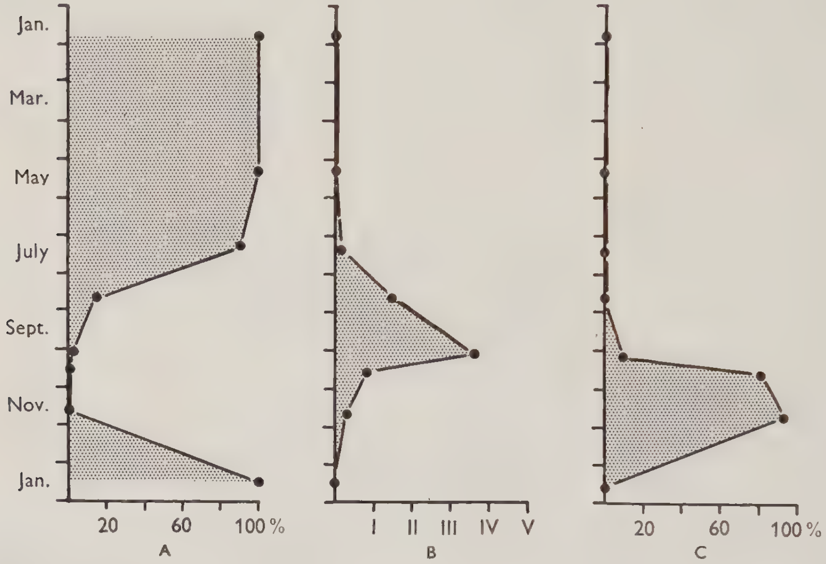
* Virgin and resting stages can be called o and N respectively, but for purposes of calculation of gonad index, both count as o. In the present investigation only the resting stage was concerned in routine work.

An example of a series of samples grouped and treated according to this scheme is given in Text-fig. 1. This method of representation brings out clearly the progression of the reproductive cycle, even when, as in *Patella*, the various stages are prolonged and tend to overlap.

ANATOMICAL RELATIONSHIPS AND HISTOLOGY OF THE GONAD

The limpets used for anatomical and histological description of the gonad stages were fixed in Bouin's fluid in sea water and stored in 70% alcohol. Normal paraffin embedding proved unsatisfactory owing to the size of the mature gonads and the large amounts of reserve material they contained. They were therefore embedded in celloidin which was subsequently impregnated with paraffin wax. Sections were cut at 5, 10, or 15 μ, and stained in either Mallory's triple stain or in Heidenhain's iron-alum haematoxylin followed by eosin or erythrosin Orange-G as a counterstain.

The gonad in both sexes lies on the ventral face of the visceral mass between this and the foot. It is unpaired and slightly displaced to the left (when viewed from above). It varies greatly in size and colour throughout the breeding season and its growth causes a good deal of displacement of other organs. For physical reasons it is unable to grow in an anterior direction, consequently its anterior margin always lies below the posterior region of the salivary glands.

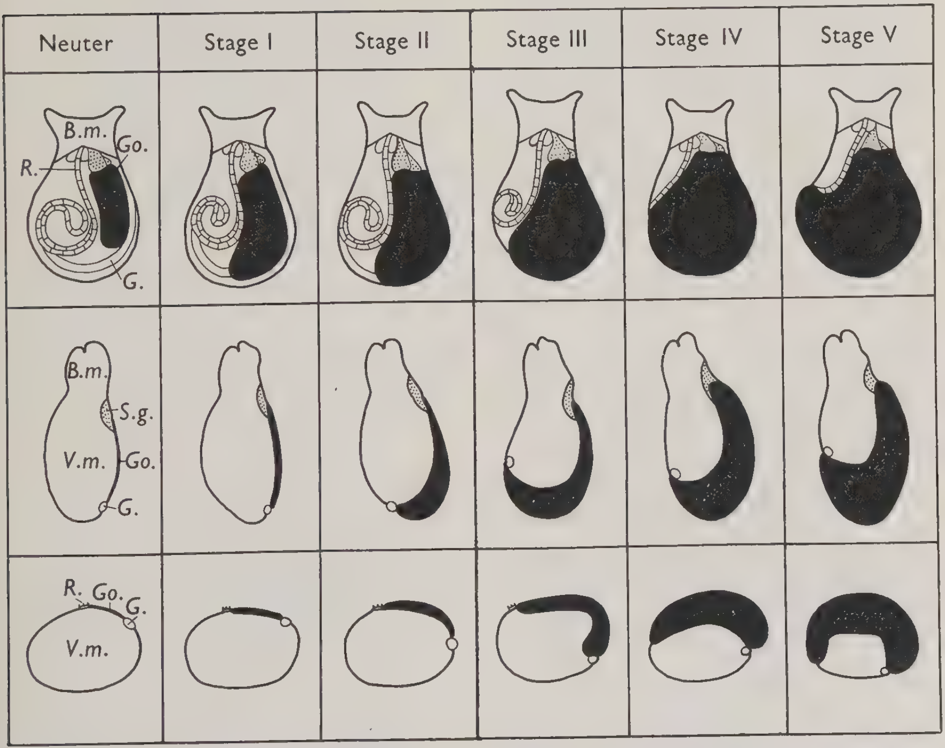


Text-fig. 1. Data defining the breeding state of a population (*Patella vulgata* from Kallow Pt., Port St Mary, Isle of Man, 1946–7; shell length 4·5–5 cm). (A) Percentage with gonad resting (neuter or stage 0). (B) Gonad index denoting the mean state of development of the population. (C) Percentage with gonad in the spawning condition.

The outer lateral margin is delimited by a loop of the hind-gut, and the inner margin by the radula. The lateral and posterior regions of the gonad undergo extensive growth, and a striking increase in thickness is noted as ripeness is approached. Text-fig. 2 shows semi-diagrammatically how the gonad increases in extent and thickness throughout its period of growth; the same diagrams also illustrate the reduction in size which occurs as spawning advances. Concomitant changes which occur in the colour of the gonad are associated largely with changes in the proportions of the reserve material. The latter is orange or brown, the pigment being probably identical with the carotenoids found in almost equal amounts in the gonads of both sexes by Goodwin & Taha (1950) and Goodwin (1950). This pigment becomes dispersed during the development of the gametes. The mature oocytes are coloured green, probably by the 'chromoprotein Y' extracted from female gonads by

Goodwin & Taha, while the increasing whiteness of the developing male gonad is related to the sperm content.

These colour changes are described below and some are illustrated in Pl. I. It must be noted that the coloured illustrations differ from Text-fig. 2 in showing the appearance of the gonad as a result of partly freeing the visceral mass from the foot and turning the latter back as far as it will go while the animal is still in the shell. This was the method used for recognizing the gonad stages in routine analysis; in practised hands it is generally satisfactory. However, as the illustrations in Text-fig. 2 show, a more accurate assessment of gonad state can be made by removing the visceral mass from the foot.



Text-fig. 2. *Patella vulgata*: semi-diagrammatic representations of arbitrary stages in the development and depletion of the gonad. Top row: gonad, visceral mass and buccal mass seen from below after removal of the foot. Middle row: longitudinal section of the buccal region and visceral mass passing through the central region of the gonad; plane of section parallel to long axis of body. Bottom row: transverse section of the visceral mass through the widest region of the gonad; plane of section parallel to short axis of body. *B.m.*, buccal mass; *G.*, gut; *Go.*, gonad; *R.*, radula; *S.g.*, salivary glands; *V.m.*, visceral mass.

The Neuter Gonad (Text-fig. 2; Pl. II, fig. 1)

In limpets of 1 cm and less in shell length the neuter gonad clearly represents the virgin state: in larger specimens which have already spawned it represents the resting spent stage.

The neuter gonad is a discrete reddish brown kidney-shaped structure. It lies on the ventral surface of the visceral mass between this and the foot. The outer and posterior extremities are delimited by a loop of the hind-gut, the anterior margin slightly overlies the salivary glands, and the inner margin lies parallel to the radula at some distance from it.

Microscopic examination of sections shows that the structure of the spent neuter gonad varies with the length of time after spawning at which it is examined. The present description applies to a gonad examined at approximately the middle of the resting period, when it consists of a very thin layer of tissue containing irregular lacunae. In addition, there are sometimes patches of relict ova or sperms, though these are never visible on macroscopic examination and may not be visible in smears. The surface layer is more obvious than in the later stages, and the underlying tissues form a loose reticulum with numerous scattered nuclei. Cell walls are difficult to distinguish and there is no germinal tissue.

Developmental Stages (Text-fig. 2)

Stage I Gonad (Pl. II, figs. 2, 6 and 7)

In the stage I gonad some growth in all dimensions has occurred and sex is recognizable from macroscopic examination. The inner margin of the gonad is contiguous with the radula and the outer and posterior margins almost reach the periphery of the visceral mass.

(*a*) *Female*. The female gonad is predominantly brown in colour though tinged with green by the developing eggs. The surface is smooth and delimited by an obvious membrane. The largest eggs are few in number and are separated by nests of developing eggs which can be seen only with a lens.

Microscopic examination of the gonad shows very few medium-sized eggs, most of which are in the central region. The gonad is seen to consist mainly of small oocytes in which vitellogenesis is well advanced, and nests of undifferentiated germ cells. The latter are attached to connective tissue trabeculae

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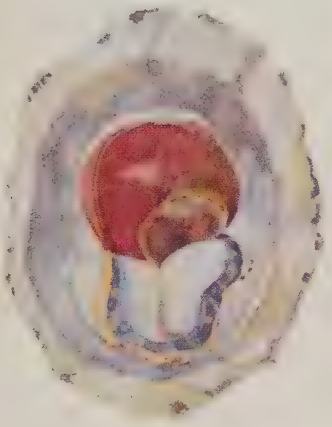
Explanation of Plate I

Patella vulgata: stages in development and depletion of the gonad showing changes in coloration. In these illustrations the *overall* colour of the ventral surfaces of the gonads is shown. The foot has been partly removed from the visceral mass and turned forward.

Fig. 1. Female; spawning stage I.
Fig. 2. Male; spawning stage I.
Fig. 3. Male; spawning stage I–II.
Fig. 4. Male; spawning stage II.
Fig. 5. Female; developmental stage V.
Fig. 6. Male; spawning stage IV.



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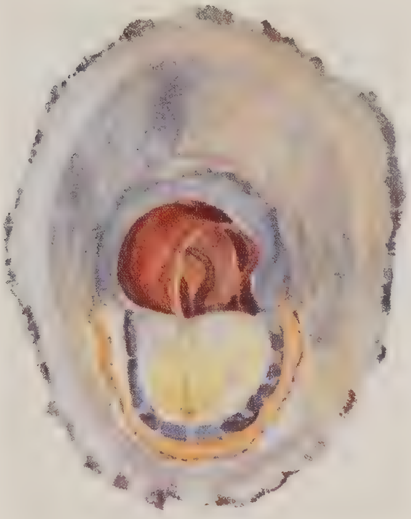

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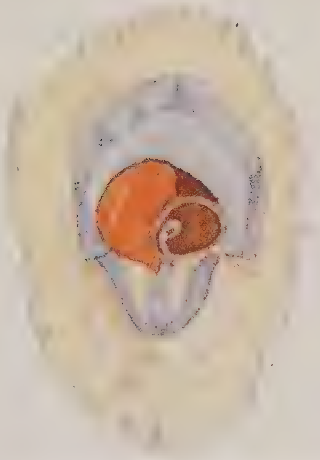

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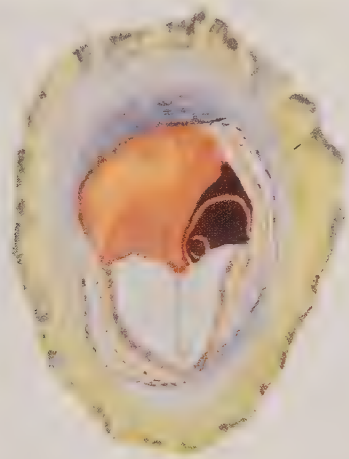

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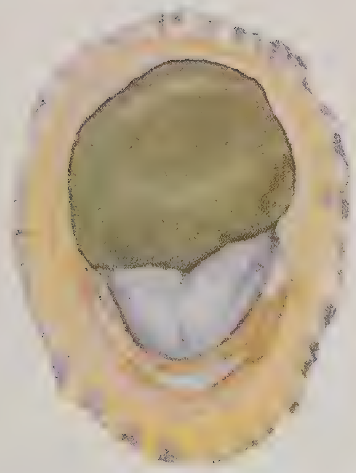

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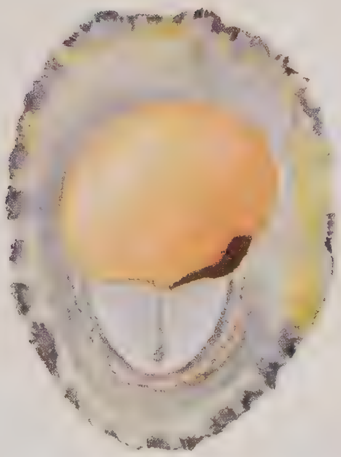

6

M. Craig pinx.

(*Facing p.* 154)

which carry the germinal epithelium into the thickness of the gonad. Interstitial tissue is small in quantity.

(*b*) *Male*. The male stage I gonad is dark yellowish brown in colour with an obvious membrane. The surface is mottled with white, the white areas representing the cavities of tubules which contain some apparently fully developed spermatozoa.

Microscopic examination shows that the seminiferous tubules of the male gonad are already well developed. The tubules extend inwards from the periphery of the gonad and consist of a connective tissue base which supports thick layers of germinal tissue consisting of spermatogonia, spermatocytes, spermatids and spermatozoa, in that order. The spermatozoa present appear fully developed and lie with their tails projecting into the lumen of the tubules. Spermatogenesis is further advanced in the dorsal regions of the gonad (i.e. those in contact with the visceral mass) as compared with the more ventral.

Stage II Gonad

By the time this stage has been reached the sexes are easily recognizable by the colour of the gonad. Growth has occurred mainly in posterior and lateral directions, and the gonad now extends over the outer and posterior margins of the visceral mass. A loop of the hind-gut still delimits the outer extremities and the inner margin lies alongside the radula.

(*a*) *Female*. The ventral surface of the gonad is smooth and glossy in macroscopic appearance, and the green colour of the eggs is already beginning to mask the brown coloration of the reserve material. There are more large oocytes visible at the surface than in stage I.

Microscopic examination of sections of the stage II gonad shows a great advance in development of the oocytes. Many are now medium to large in size and the 'chorion' layer, which is strongly eosinophil, is a very obvious feature of each egg. Connective tissue trabeculae are well developed and the germinal layer which they support shows representative stages in oogenesis.

(*b*) *Male*. The surface of the male stage II gonad shows obvious mottling due to the germinal tubules which reach out to the periphery of the gonad. The peripheral regions of the tubules are brown in colour, whereas the central lumen, full of spermatozoa, is milky white. The arrangement of the tubules gives to the gonad surface an appearance strongly reminiscent of the surface of the mammalian brain, though smooth.

Microscopic examination shows that the proportion of spermatozoa to earlier stages in spermatogenesis is considerably higher than in stage I. Spermatid nuclei are also a prominent feature of the tubule walls. Nuclei which do not stain strongly in Heidenhain's haematoxylin (those of the earliest stages in spermatogenesis), are almost entirely limited to the peripheral regions of the tubules. Active spermatozoa can be obtained from stage II male gonads.

Stage III Gonad (Pl. II, figs. 3, 8)

The stage III gonad shows a considerable increase in thickness when compared with stage II and, also, marked growth of its posterior regions. These have now extended on to the dorsal face of the visceral mass and form a thick extension of its posterior extremity.

(*a*) *Female*. The brown colour of the early gonad is now greatly masked by the green eggs. In some specimens the latter are olive-green, whereas in others they are a bright bluish green: several intermediate shades are also encountered. The eggs at the ventral surface of the gonad show marked differences in size.

The stage III gonad is not greatly different in microscopic appearance from the stage II gonad. There are still many small oocytes present, especially on the trabeculae which ramify throughout the gonad. The proportion of large eggs at the surface is slightly higher than in stage II.

(*b*) *Male*. The stage III male gonad is yellowish brown mottled with white. The white areas become more extensive as the ripe spermatozoa increase.

Microscopic examination shows a higher proportion of fully developed spermatozoa than in the stage II gonad. The walls of the seminiferous tubules consist mainly of spermatozoa and spermatids, though their peripheral regions contain considerable amounts of reserve material and nests of differentiating germ cells. The difference in ripeness of dorsal and ventral regions of the gonad is now less marked, though the proportion of fully developed spermatozoa in the dorsal region is still somewhat higher.

Stage IV Gonad

In the stage IV gonad the increase in thickness is yet more marked. This is especially true of the posterior region which now extends well beyond the

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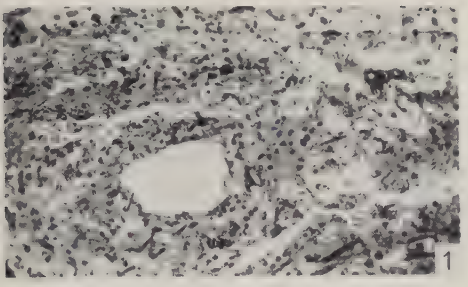
Explanation of Plate II

Patella vulgata: Photomicrographs of sections through male and female gonads in various stages of development and depletion. The plane of section is parallel to the dorso-ventral axis of the gonad. Excepting Figs. 1 and 6, the photographs show part of the ventral surface of the gonad and the area immediately adjacent to it. Preparations stained with Heidenhain's haematoxylin and eosin.

Fig. 1. Neuter gonad. Traces of tubule structure still evident. Note small groups of relict sperms. × 180.
Fig. 2. L.S. ovary; stage I (developing). × 66.
Fig. 3. L.S. ovary; stage III (developing). × 66.
Fig. 4. L.S. ovary; stage V (developing). × 66. Note 'interstitial' tissue.
Fig. 5. L.S. ovary; stage III (spawning). × 66.
Fig. 6. L.S. seminiferous tubule from stage I male (developing). × 300.
Fig. 7. L.S. testis; stage I (developing). × 66. Note salivary gland tissue in lower right-hand corner.
Fig. 8. L.S. testis; stage III (developing). × 66.
Fig. 9. L.S. testis; stage V (developing). × 66.
Fig. 10. L.S. testis; stage III (spawning). × 66.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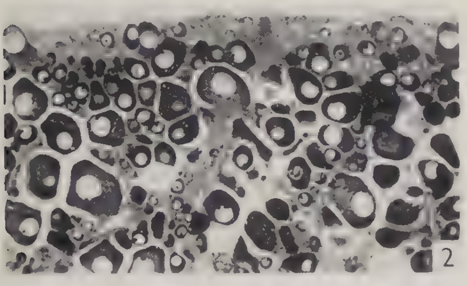
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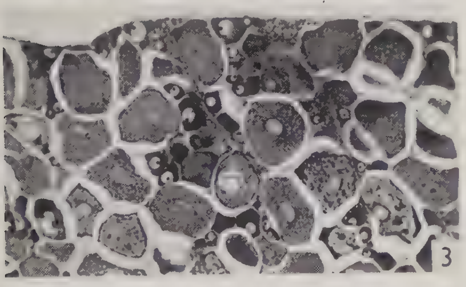
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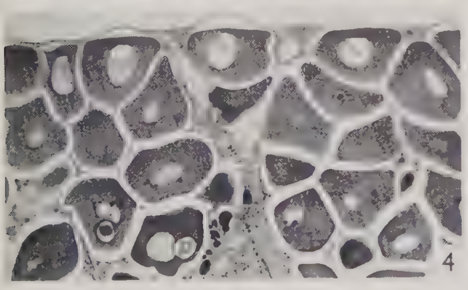
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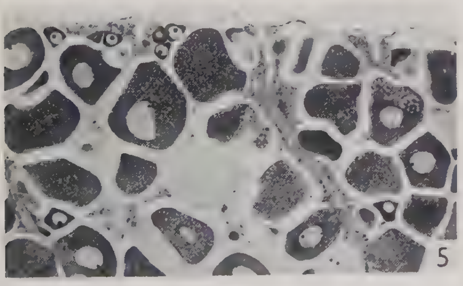
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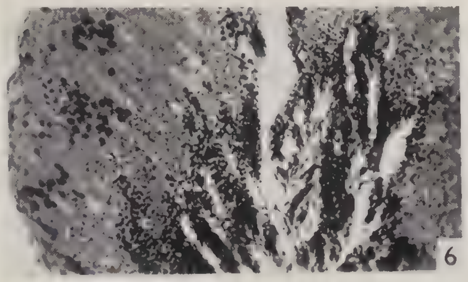
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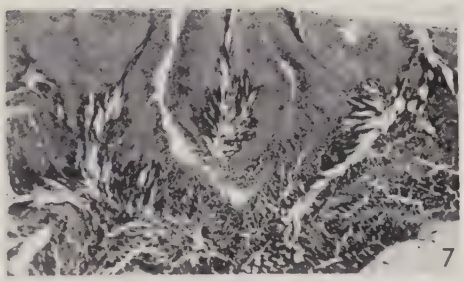
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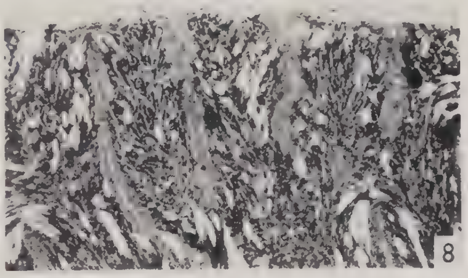
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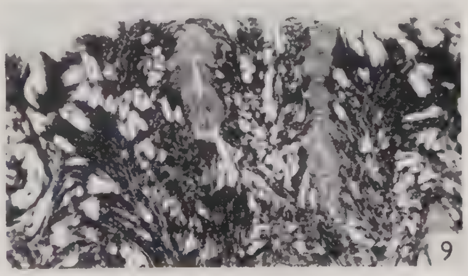
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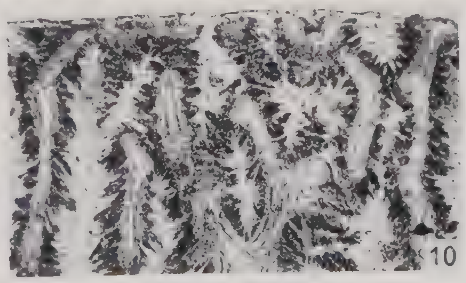
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limit of the rest of the visceral mass. Some extension of the lateral margins of the gonad has also occurred, and most of the radula has now been displaced on to the lateral face of the visceral mass.

(*a*) *Female*. All the eggs visible to the naked eye on the ventral surface of the stage IV gonad are large and uniform. Different individuals show marked differences in colour of the gonad, the commonest colour varieties being bluish green, olive green and olive brown.

Examination of sections shows that the great majority of the surface eggs are now large, though there are a few small oocytes attached to the trabecular tissue. Interstitial tissue is small in amount, being mainly represented by islands of tissue consisting of groups of cells having prominent nuclei, but in which cell boundaries are difficult to identify.

(*b*) *Male*. The colour of the stage IV male gonad is predominantly cream, though there are traces of the orange-brown mottling which represents reserve material in the peripheral regions of the seminiferous tubules.

Microscopic examination shows that fully developed spermatozoa are the most prominent constituent, though a few spermatids and earlier stages are present at the periphery of the tubules.

Stage V Gonad (Pl. I, fig. 5; Pl. II, figs. 4, 9)

The stage V gonad is of such a size that it exerts considerable pressure on the tissues which attach the margin of the visceral mass to the foot, and the operation of removing a fully ripe limpet from the rock is often sufficient to tear foot and visceral mass apart. When the visceral mass is partly removed from the foot and turned forward, the entire structure is seen to be overlaid by gonad. To allow for this great increase in gonad volume, the progressive shrinkage in size of the visceral mass, which is noticeable even in the stage III gonad, now reaches a maximum. Although no detailed observations have been made, it appears that feeding either stops or slows down and the gut virtually empties. In this way, since the gut accounts for a considerable part of the volume of the visceral mass, the gonad is allowed to grow in a space of limited volume. The posterior and lateral extension of the gonad which is also a feature of the earlier stages, is maximal in the stage V gonad.

(*a*) *Female*. The stage V female gonad shows a similar range of coloration to stage IV, from which it differs mainly in size. The surface eggs are large and uniform in size and appear loose and ready for shedding.

Microscopic examination of sections shows that the gonad is an almost uniform mass of large eggs in which only traces of the original trabeculae remain and there are very few developmental stages. Each egg is invested by a very thin 'chorion' which, in most preparations, has become separated from the surface of the egg in consequence of the histological procedures employed. There are evident islands of interstitial tissue consisting of masses of small cells with densely staining nuclei. These islands are characteristic of all the later developmental stages: their function is unknown.

(*b*) *Male*. The overall colour is whitish cream, the brown mottling being largely masked. Surface patches are commonly found which are white in colour and these are believed to represent areas in which spermatozoa are being liberated prior to shedding.

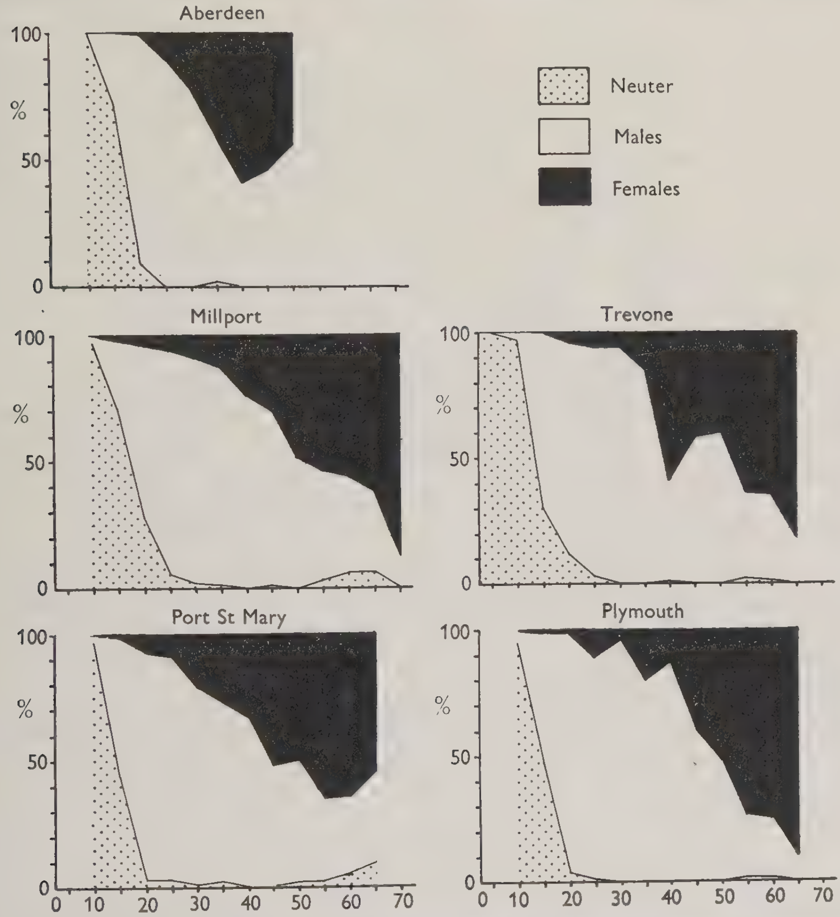
Microscopic examination shows an almost uniform mass consisting mainly of fully developed spermatozoa. The majority of these are attached to the germinal epithelium. A considerable proportion of spermatids is still present, though earlier developmental stages are uncommon. The peripheral areas of the gonad frequently show irregular lacunae.

Spawning Stages (Pl. I; Pl. II, figs. 5, 10)

Gonads undergoing regression are subdivided in the same manner as are the progressive developmental stages. Thus a gonad in which spawning has occurred to the extent that it is morphologically similar to developmental stage III is classified as spawning stage III.

Spawning gonads are difficult to distinguish from developing gonads by any morphological criteria. Early spawning stages in females can be distinguished to some extent by the appearance of the surface eggs: as spawning advances they appear much more loosely packed than in the corresponding developmental stages and, in addition, they are more uniformly large. They appear more rounded and seem to be detached from the substratum of the gonad as though ready for shedding. Corresponding stages in development and regression are slightly different in colour, the proportion of the brown non-germinal tissue being higher in the latter. Microscopic examination of sections shows more striking differences, though this method of study would be less practicable for the large-scale investigation of the breeding cycle. The uniformity of the eggs and the pronounced gaps between them, occupied by a loose chromophobe connective tissue, are highly characteristic features (Pl. II, fig. 5). However, considerable numbers of small oocytes are often present in the spawning gonad, since spawning frequently alternates with subsidiary bursts of development. In spawning males, the gonad frequently shows white patches in which the tubules appear to have broken down and, furthermore, the colour of the brown non-germinal tissue changes from orange-brown to a darker brown and becomes progressively greater in relative amount as spawning advances. Sections of male gonads in which spawning is far advanced have a highly characteristic appearance. The main constituent is the chromophobe non-germinal tissue: fully developed spermatozoa are few in number and limited to the free edges of the tubules. Earlier stages in spermatogenesis are practically non-existent.

Thus the criteria that exist for separating spawning individuals from those which are developing are undoubtedly somewhat subjective and difficult to distinguish and describe: they become recognizable only as a result of considerable experience in examining gonads throughout the breeding season.



Text-fig. 3. *Patella vulgata*: the proportions of sexes at different shell lengths (5 mm groups) in samples from several localities, 1945–6.

SEX PROPORTIONS

Approximately 1000 individuals were examined from each of the localities Aberdeen, Millport, Port St Mary, Trevone and Plymouth, between September 1945 and February 1946. As far as possible about 100 specimens were examined in each 5 mm size-group. The actual numbers of males, females, and neuter individuals in each size-group is given in Table II, while their distribution as a percentage of the total number of specimens examined is

TABLE II. THE PROPORTIONS OF SEXES IN *PATELLA VULGATA*

(Samples from several localities during the period September 1945 to February 1946, grouped according to the length of shell as an approximate indication of age.)

Locality	Sex	Shell length (mm) 0–5	6–10	11–15	16–20	21–25	26–30	31–35	36–40	41–45	46–50	51–55	56–60	61–65	66–70	Totals
Aberdeen	Neuter	—	59	76	10	—	—	2	—	—	—	—	—	—	—	147
	Male	—	—	30	102	218	168	58	53	44	9	—	—	—	—	682
	Female	—	—	—	1	24	48	42	76	52	7	2	—	—	—	252
Millport	Neuter	—	28	90	34	6	2	1	—	1	—	3	11	5	—	181
	Male	—	1	34	82	87	104	97	77	70	57	49	74	29	1	762
	Female	—	—	2	5	6	11	15	24	30	55	61	110	56	7	382
Port St Mary	Neuter	—	96	74	6	3	1	1	—	—	1	2	3	1	—	188
	Male	—	3	94	206	76	55	37	22	20	28	26	20	4	—	591
	Female	—	—	1	15	7	15	14	11	22	29	53	41	6	—	214
Trevone	Neuter	3	135	30	28	6	—	—	1	—	—	3	1	—	—	207
	Male	—	4	68	206	164	140	92	42	98	86	56	30	2	—	988
	Female	—	—	—	9	10	9	16	61	71	57	104	66	9	—	412
Plymouth	Neuter	—	101	82	4	—	—	—	—	—	—	1	1	—	—	189
	Male	—	5	90	105	84	115	83	73	28	42	27	32	1	—	685
	Female	—	—	1	1	10	5	21	11	25	46	81	100	9	—	310

shown in Text-fig. 3. As has been known for some time (Orton, 1920, 1928) the smaller sizes of limpet in which sex can be determined are almost entirely male, while the larger sizes are preponderantly female. In the range up to 10 mm shell length practically all specimens examined in the present study were neuter, but between 16 and 25 mm at all localities there were at least 90% males. The two sexes were approximately equal in number in specimens having a shell length around 40 mm, while 60–70% of those specimens having a shell length of 60 mm were female (such large specimens were not found in the Aberdeen samples). In limpets having a shell in excess of 60 mm even higher proportions of females were sometimes found (Millport, Trevone, Plymouth), but this may have been due to the small numbers examined.

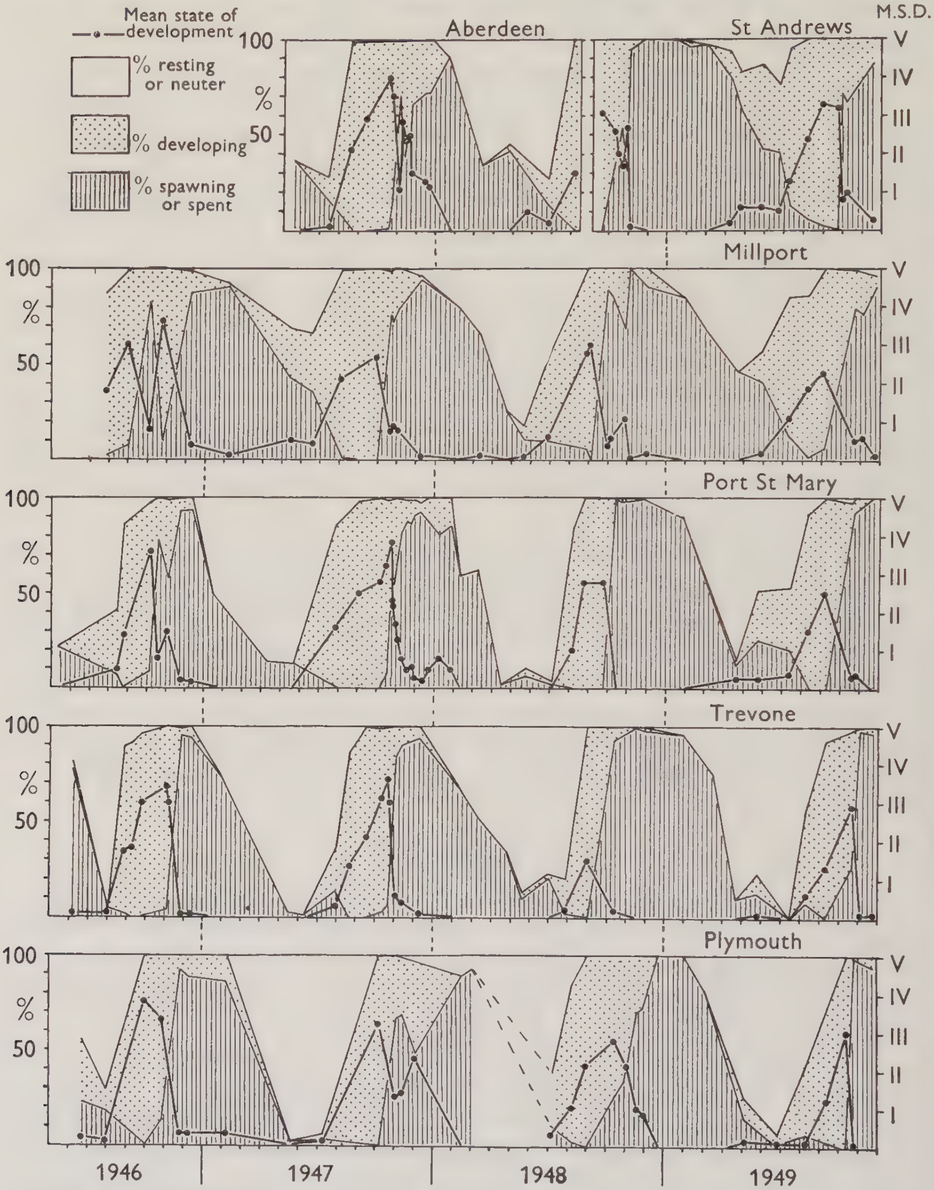
These changes in sex proportions with length can be explained in two ways. If one assumes that the rates of growth and development of males and females are similar, and this is certainly true for the gonad, it can be suggested that almost all individuals start their breeding life as males, but that later in life a large proportion of them become female. On the other hand, if one assumes that the rate of growth and development may differ markedly in the two sexes, the preponderance of females in the larger size-groups can be explained by differential mortality, more than 90% of the males dying before they reach a shell length of 60 mm.

Of these explanations, that of change of sex is the simpler and more acceptable, since the phenomenon occurs in many other molluscs. Sex-reversal in such an animal does not involve major anatomical changes since there are no secondary sexual characters. The long resting phase of the gonad, when the sexes are not discernible, and the general similarity of the breeding cycle in male and female makes sex change very difficult to detect. No direct evidence is yet available, but we will return to the problem when considering the other species of *Patella* which show very different sex proportions.

THE BREEDING CYCLE

The preliminary observations showed that collection of individuals from 30 to 60 mm shell length would provide roughly equal numbers of males and females. Regular samples of these sizes were obtained from each locality, at monthly intervals during the relatively inactive periods and more frequently about the time of spawning.

At first, samples of 100 were examined, but it was later found to be possible to reduce this number by half without affecting the results. From Millport, Port St Mary, Trevone and Plymouth, sampling was maintained from the spring of 1946 to the end of 1949. The Aberdeen samples were insufficient in 1946 and 1947, and the series was discontinued in 1949, being replaced by the St Andrews samples, not begun until late in 1947. The results from these samples are given in Tables IV–IX (Appendix) as numbers of individuals found in each gonad stage in the separate sexes, and in the neuter or resting stage. The four variables used to define the breeding state of the population (p. 4) have been extracted from these tables, and are shown in Text-fig. 4.



Text-fig. 4. *Patella vulgata*: the breeding cycle of samples from several localities, 1946–9, showing the percentages with gonad resting, developing, and spawning. The thick line gives the mean state of development of the gonad (ordinates on right side).

From the Tables and Text-fig. 4 it can be seen that *P. vulgata* has a long resting phase: during the spring and early summer the animal is effectively neuter. This phase must last up to 9 months in some individuals, and for over 3 months in 50% of the population, except at St Andrews, where it is rather short. The resting phase is followed by a much shorter period of development from June to October, not usually more than 3–4 months, during which the gonad increases steadily in size. There may be a further period of up to 1 month during which little change takes place in the samples. At this stage the gonad is apparently fully mature, and a high proportion of viable larvae may be obtained by artificial fertilization. Some time between the beginning of September and the end of October, according to year and locality, a comparatively rapid change from the developing to the spawning condition takes place. Finally, the proportion of spawning and spent individuals declines slowly and regularly between December and June, as more and more pass into the resting condition.

This cycle is subject to some variation from year to year and variation is also shown between the different localities. Only at the more northerly stations (Aberdeen, St Andrews, Millport; cf. Text-fig. 4) did the spawning and developing phases overlap: in the other localities a higher proportion of the limpets passed into the resting phase together, while the periods of development and spawning were shorter. This suggests that temperature may influence the resting period, and that the emptying of the gonad, and its change to the resting phase is speeded up by the warmer conditions in the south. Temperature differences may also explain the fact that gonad development starts earlier in the more northern localities, in which a longer development period may be necessary because of the lower environmental temperatures. However, temperature differences can hardly account for the variations in the exact time of onset of spawning at the different stations, since, of the years studied, only 1949 and 1952 showed a significant difference between the northerly and southerly localities in this respect.

SPAWNING

The dates between which spawning started and those on which most (at least 90%) of the limpets in the samples were in the spawning condition are given in Table III. This table includes information for some other stations, in addition to those regularly sampled, and for 1952, when only the spawning period was studied. More precise information for the regular samples will be found in the Appendix Tables IV–IX, while Text-fig. 4 shows diagrammatically the onset of spawning.

In some years, notably 1947 and 1948, spawning seems to have started practically simultaneously at all stations towards the end of October. At Plymouth (both stations) spawning again began in October in 1946, 1949 and

TABLE III. THE SPAWNING PERIOD OF *PATELLA VULGATA* AT SEVERAL BRITISH LOCALITIES IN 1947–49 AND 1952

	1946		1947		1948		1949		1952	
	Dates between which spawning began	Date at which 90 % of sample were spawning	Dates between which spawning began	Date at which 90 % of sample were spawning	Dates between which spawning began	Date at which 90 % of sample were spawning	Dates between which spawning began	Date at which 90 % of sample were spawning	Dates between which spawning began	Date at which 90 % of sample were spawning
Aberdeen	—	—	9 Sept. to 17 Oct.	19 Jan. 1948	7 Sept. to 10 Oct.	6 Dec.	—	—	—	—
St Andrews	—	—	Before 3 Nov.	5 Nov.	17 Sep. to 18 Oct.	2 Nov.	22 Sept. to 30 Sept.	Not in 1949	21 to 27 Sept.	4 Oct.
Millport	—	—	1 Oct. to 18 Oct.	9 Dec.	30 Sept. to 2 Nov.	2 Nov.	10 Aug. to 3 Sept.	24 Nov.	Before 2 Oct.	Before 2 Oct.
Port St Mary, Isle of Man	21 Aug. to 3 Sept.	25 Nov.	6 Oct. to 22 Oct.	20 Nov.	24 Sept. to 13 Oct.	13 Oct.	5 Sept. to 17 Oct.	23 Oct.	Before 3 Oct.	24 Oct.
Other places on east side of Isle of Man	—	—	1 Oct. to 5 Oct.	21 Nov.	24 Sept. to 25 Sept.	25 Oct.	5 Sept. to 20 Oct.	—	—	—
West side of Isle of Man	—	—	15 Oct. to 28 Oct.	—	6 Sept. to 22 Oct.	22 Oct.	—	—	—	—
Trevone	24 Sept. to 2 Nov.	25 Nov.	17 Sept. to 11 Oct.	14 Nov.	30 Aug. to 13 Oct.	13 Oct.	10 Sept. to 19 Sept.	4 Nov.	Before 3 Oct.	Before 3 Oct.
Plymouth Breakwater	1 Oct. to 28 Oct.	27 Nov.	6 Oct. to 3 Nov.	16 Feb. 1948	1 Sept. to 14 Oct.	21 Dec.	12 Oct. to 27 Oct.	27 Oct.	22 Oct. to 4 Nov.	4 Nov.
Plymouth, Rum Bay	—	—	—	—	1 Sept. to 14 Oct.	23 Nov.	12 Oct. to 4 Nov.	4 Nov.	—	—

1952, but elsewhere occurred up to a month earlier. Practically all stations reached peak spawning (over 90%), in spite of this, some time in November.

Details of spawning can only be inferred from the samples, since actual 'running' specimens were rarely seen, although a slow release of eggs was obtained from later spawning stages in the laboratory. The first indication of spawning in the samples was usually a fairly sudden decline in gonad size, but after this, liberation of eggs and sperm must have continued slowly, for 2–4 weeks passed before the first fully spent (stage I) individuals appeared. Thus the rapid swing from the developing to the spawning condition which frequently occurred must be distinguished from the swift and complete voiding of gametes that can take place in some other organisms. The spawning of the population as a whole was also slow compared with some other organisms, and it was sometimes 6 months or more (10 months in the more northern localities) after the start of spawning before all specimens had passed into the resting or developing phases.

Although the peak of spawning occurred from October to March at all localities, and thus *Patella vulgata* is to be regarded as a winter spawning species in Britain, the tables and figures show that some spawning can occur from September to the following June. In the more northerly stations, in certain years, the spawning season may be very extended. In June 1949, specimens of *P. vulgata* containing ripe genital products were still fairly plentiful at St Andrews (over 40% spawning stages in sample) and an artificial fertilization was made from which larvae were reared through metamorphosis (Dodd, 1956). It must therefore be stressed that gonad examination over a period of more than 1 year is necessary before any decision can be made about the breeding periods of limpets and related gastropods: little reliance can be placed on a few isolated samples. Records of the occurrence of the larvae in the plankton, unless made at frequent intervals over a long period, may also have little value.

Spawning Stimuli

The rapidity of the onset of spawning is to some extent a gauge of the strength of the supposed stimulus that induces spawning. For example, in 1946 at Trevone, in 1947 at Millport, Port St Mary, and Trevone, in 1948 at Millport and Port St Mary, and in 1949 at Plymouth over 90% of the samples were spawning or spent within a month after the first spawning specimens were found. Presumably on these occasions the stimulus to spawn was stronger than at other times. The stimulus can hardly be internal, since in some years the whole population at different localities was involved within a few days, while in other years there were differences between almost adjacent populations (e.g. compare Plymouth breakwater and Plymouth, Rum Bay, in 1948, and the east and west coasts of the Isle of Man in 1947; Table III). At the same time the onset of spawning can hardly be due to some climatic or hydrographic factor such as temperature or the nature of the water masses, since spawning may start simultaneously in different areas. The spawning stimulus does in fact appear to be capable of over-riding the breeding-cycle; for example, at Trevone in 1948, it appears to have arrived relatively early, for spawning began while the mean state of development of the gonad was low. Quite frequently spawning occurs while this index is at III or IV.

These differences appear to preclude any relation between spawning and the phases of the moon such as may occur in other organisms (e.g. *Ostrea*, Orton, 1926, 1927; *Arenicola*, Newell, 1948). Samples were not taken at sufficiently close intervals for possible lunar periodicity in spawning to be shown with any accuracy, except for the series from Port St Mary and Trevone in 1947. These particular samples show little lunar influence; the main spawning stimulus seems to have arrived between 22 and 25 October, some 12 days behind the full moon, and during a period of neap tides. Samples taken at less frequent intervals in other years, e.g. at Plymouth in 1949 and at Port St Mary in 1948, also suggest that any correlation between the moon and spawning lay with a period of neap tides; but the extent of such correlation was slight.

There is much better indication of a relation between wave-action or on-shore winds and the start of spawning. For example in 1947 spawning began at Trevone between 17 September and 11 October, and continued very weakly (not more than 7% spawners) up to 21 October. During this period some rough seas may have been experienced since on a few days winds at St Eval, North Cornwall, (Air Ministry, 1947) reached 21 knots between south-west and north. A very strong stimulus to spawning appears to have been in operation between 21 October and 4 November, by which date over 80% of the samples were in the spawned condition. This strong stimulus coincided with the occurrence on six of the days of winds of 21 knots and over between south and west at St Eval, and on four of these days the wind reached 30 knots or over. In the same year at Port St Mary the initial stimulus also appears to have been weak, and may have been associated with winds of up to 21 knots (once 27) recorded at the Pt. of Ayre, I.O.M. (Air Ministry, 1947) from south-east to west between 6 and 22 October. The major spawning stimulus seems to have arrived between 22 and 25 October, when the wind (recorded at the Pt. of Ayre) sometimes reached 21 knots from the south-east. This is the critical wind sector for the Port St Mary collecting station, and causes maximum wave action there.

At the other localities in 1947, the onset of spawning was much more gradual.

An extremely rapid swing-over to the spawning condition occurred at Port St Mary in 1948 between 24 September and 13 October, a period of almost continuously high winds and rough seas around the Isle of Man. Thus the winds recorded at the Pt. of Ayre (Air Ministry, 1948) reached 21 knots between south-east and west on 8 days, and over 30 knots on 3 days, while from personal observations the seas at Port St Mary were very rough.

At the other localities conditions were much calmer in 1948. At Plymouth the wind rarely exceeded 16 knots during September and October, and it is therefore interesting that this was a year in which spawning progressed very slowly there.

In 1949 the change to the spawning condition was more rapid at Trevone and Plymouth than at the other stations. At St Andrews and Port St Mary the initial spawning seems to have coincided with the occurrence of winds of up to 21 knots about 21–22 September, from the north-east at Leuchars, near St Andrews (Air Ministry, 1949) and from the south-east at Ronaldsway Airport, near Port St Mary. There was little wind at Plymouth during this period. The maximum change to the spawning condition at Plymouth and Trevone took place from 12 October to the end of the month, and seems to have coincided with winds of 21–30 knots from the south and west between 15 and 22 October, and similar winds from south through west to north between 22 and 26 October, at Plymouth and St Eval (Air Ministry, 1949).

The change to the spawning condition was even more rapid at St Andrews in 1952, between 21 and 27 September; the whole population was spent by 4 October. Winds of over 20 knots, sometimes 30 knots, were recorded practically every day during this period from directions between west and north at Leuchars (Air Ministry, 1952), and rough seas must have been fairly continuous. At Port St Mary spawning appears to have started about the same period, when rough seas were observed practically continuously from 23 to 28 September, and the winds at Ronaldsway Airport exceeded 20 knots, and sometimes reached 30 knots, from directions between south and north-west (Air Ministry, 1952). At Plymouth spawning was later. Rough seas were observed on only 3 isolated days in September, and spawning seems to have corresponded with rough seas on 25 to 27 October, and winds of 22–35 knots from south to west from 25 to 29 October.

Thus in 1947–49 and 1952 the initiation of spawning and periods of rapid changeover to the spawning condition coincided with periods of strong onshore winds and rough onshore seas. On some occasions also, as mentioned above, the greatest changeover to the spawning condition coincided with neap tides. If wave-action or onshore winds are major factors stimulating spawning, their effect would be enhanced during neap tides, when the smaller tidal range and slower movement would prolong their influence on the limpet habitat.

If the phenomena of wave-action or onshore winds and spawning are related, it becomes important to consider how the relationship is mediated. The direct effect of mechanical shock is an obvious factor to consider (cf. Chipperfield, 1953). It might either trigger spawning in the majority of individuals in a population, or else induce it in the ripest members which in turn might stimulate the remainder through a chemical effect of the gametes or other substances released on spawning. It may also be that some chemical or biological factor which induces spawning is brought inshore by turbulence. These and other possibilities have been investigated by an extensive series of laboratory experiments which have failed to show effects of the kind postulated. However, it must be admitted that experiments of this sort are hard to design satisfactorily and extremely difficult to carry out in the laboratory and the negative results must be interpreted accordingly.

The problem of spawning stimuli has been studied in detail in the other species of *Patella*, and will be discussed further in a later paper.

COMPARISON WITH OTHER ANIMALS

The breeding cycle which we have described for *Patella vulgata* contrasts markedly with the cycle in the lamellibranch molluscs, the reproductive processes of which proceed more rapidly. For example, in *Ostrea* (Orton, 1926, 1927, 1936), and in *Pecten* (Mason[1]), which breed during the summer in Britain, the gonad empties rapidly, and sometimes two batches of gametes can be matured before the autumn. In the former species, although only one batch of eggs is produced by a female, a high proportion of the females switch rapidly to the male phase and start sperm production immediately after spawning. Even in intertidal *Mytilus* (Chipperfield, 1953) the act of spawning, both of individuals and of the population as a whole, is much more rapid than in *Patella vulgata*. The same applies to most fish with planktonic larvae (e.g. *Clupea*, Orton, 1916), though in some (e.g. *Gadus merlangus*, Bowers, 1954), the spawning period of the population may be as extensive as that of *Patella*.

The resting period in *P. vulgata* seems unduly extended when compared with the duration of the similar stage found in some lamellibranchs (Chipperfield, 1953) and fishes (Orton & Jones, 1940; Jones, 1940). In fact it may be said that the predominant feature of the breeding of *P. vulgata* is the slow progression of the cycle. Since it is possible that this slow progression is connected with winter breeding, it is instructive to compare *Patella* with another intertidal animal which starts to spawn at about the same time of the year, the lugworm *Arenicola marina*. Although the period of development of the eggs and sperm, June to October or November (Newell, 1948; Southward, 1953), is similar to that of *Patella vulgata*, the gametes are voided much more rapidly in *Arenicola*: this means that the resting period is correspondingly longer than in *Patella vulgata*. It may be that animals with a northern distribution, which spawn in late autumn or winter, are characterized by a long resting period.

Some investigations which are being made by one of us (A. J. S.) on the breeding of certain common intertidal top-shells indicate that *Osilinus lineatus* and *Gibbula umbilicalis* have a breeding cycle very similar to that of *Patella vulgata*, although the resting phase may be shorter. As these animals occupy very similar habitats, and have habits similar to those of limpets, it seems possible that the slowness of the breeding cycle, and in particular the slow progression of spawning, is coupled with the mode of life. If the rate of growth is any guide (Fischer-Piette, 1941) the vital activities of limpets, especially at the higher levels on the shore, proceed at a very slow rate: one might therefore expect that the breeding cycle in such animals would progress slowly.

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[1] J. Mason. 'Investigations on the scallop (*Pecten maximus* L.) in Manx waters.' Univ. Liverpool, Ph.D. Thesis, 1953.

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SUMMARY

Observations have been made on the breeding of *Patella vulgata*, by examination of regular samples from six localities in Britain over a period of 5 years.

The gonad stages were examined and grouped according to an arbitrary scheme, the advantages of which are discussed. Criteria are given by which the arbitrary stages can be distinguished, and their anatomy and histology described.

P. vulgata is shown to be a winter breeder in Britain. The gonad develops from June to September, and the peak of spawning occurs between October and December. Subsidiary spawning may take place at any time between September and June. From January to June the gonads pass into a resting phase. The length of the resting phase, and the proportion of individuals resting at any one time may be related to the temperature of the locality.

The main period of spawning varies from year to year and from place to place. It is not obviously related to temperature, tides, nor to the phases of the moon. The only factors with which spawning appears so far to be correlated are rough seas and onshore winds. Spawning may therefore be initiated either by physical shock, or some factor brought inshore from deeper water, but all attempts to investigate the phenomenon experimentally have failed.

In comparison with other molluscs, *P. vulgata* has a much longer resting phase, but it seems possible that this is characteristic of some winter-breeding intertidal animals.

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APPENDIX

TABLE IV. DISTRIBUTION OF GONAD STAGES IN SAMPLES OF *PATELLA VULGATA* FROM ABERDEEN

		Male									Neuter	Female								
		Developing					Spawning					Developing					Spawning			
Date	No. in sample	I	II	III	IV	V	IV	III	II	I		I	II	III	IV	V	IV	III	II	I
947																				
Feb.	116	—	—	—	—	—	—	—	2	23	63	—	—	—	—	—	—	—	—	28
May	97	—	—	—	—	—	—	—	—	15	62	—	—	—	—	—	—	—	—	20
July	96	10	2	1	—	—	—	—	—	—	68	1	—	—	—	—	—	—	—	14
Aug.	113	2	30	17	4	—	—	—	—	—	1	26	31	2	—	—	—	—	—	—
Sept.	98	1	6	19	7	—	—	—	—	—	—	7	20	31	7	—	—	—	—	—
Oct.	123	—	3	10	25	13	—	1	—	—	1	—	5	13	32	20	—	—	—	—
Oct.	118	—	—	6	31	13	1	7	—	—	—	—	—	8	27	13	—	9	3	—
Nov.	134	—	2	—	4	1	8	26	26	—	—	—	3	4	18	7	—	3	22	10
Nov.	103	—	—	12	22	10	3	11	5	—	—	—	—	—	17	10	1	8	2	2
Nov.	110	—	—	4	—	12	9	10	2	2	—	—	2	4	10	26	10	10	8	1
Nov.	104	—	—	1	3	15	5	7	4	1	—	—	—	1	1	33	10	13	8	2
Nov.	121	—	—	2	1	5	18	21	2	—	1	—	—	—	3	25	11	24	7	1
Dec.	101	—	—	2	—	4	7	22	5	—	—	—	1	—	5	16	14	12	12	1
Dec.	104	—	1	5	2	5	5	34	5	—	—	—	1	2	5	8	12	9	9	1
948																				
Jan.	98	—	—	—	—	—	—	—	8	35	9	—	—	—	—	—	—	—	—	46
Feb.	69	—	—	—	—	—	—	—	—	27	27	—	—	—	—	—	—	—	—	15
Mar.	63	—	—	—	—	—	—	—	—	17	41	—	—	—	—	—	—	—	—	5
Apr.	62	2	—	—	—	—	—	—	—	13	34	—	—	—	—	—	—	—	—	13
May	57	3	—	—	—	—	—	—	—	1	49	—	—	—	—	—	—	—	—	4
June	74	10	—	—	—	—	—	—	—	1	54	—	—	—	—	—	—	—	—	9
Aug.	62	13	10	1	—	—	—	—	—	—	—	36	2	—	—	—	—	—	—	—
Sept.	63	2	16	6	—	—	—	—	—	—	—	12	20	6	1	—	—	—	—	—
Oct.	67	—	—	4	2	—	—	14	16	—	—	—	1	4	2	1	1	10	12	—
Oct.	56	—	9	9	2	—	—	4	1	—	—	—	5	8	—	—	—	5	12	1
Oct.	71	—	3	13	2	—	—	5	18	4	—	—	5	3	1	—	—	8	8	1
Nov.	76	—	4	1	1	—	—	—	5	23	—	—	—	2	—	—	—	3	7	30
Dec.	64	—	1	1	—	—	—	2	12	18	—	—	—	1	—	—	—	6	4	19
Dec.	59	—	—	—	—	—	—	—	—	27	—	—	—	—	—	—	—	—	—	32
949																				
Jan.	71	—	—	—	—	—	—	—	—	34	6	—	—	—	—	—	—	—	—	31
Feb.	66	—	—	—	—	—	—	—	2	31	5	—	—	—	—	—	—	—	—	28
Apr.	64	—	—	—	—	—	—	—	—	15	20	—	—	—	—	—	—	—	—	29

TABLE V. DISTRIBUTION OF GONAD STAGES IN SAMPLES OF *PATELLA VULGATA* FROM ST ANDREWS

Date	No. in sample	Male									Neuter	Female								
		Developing					Spawning					Developing					Spawning			
		I	II	III	IV	V	IV	III	II	I		I	II	III	IV	V	IV	III	II	I
1947																				
3 Nov.	105	—	1	3	17	10	4	6	2	—	—	—	3	5	29	20	1	1	3	—
5 Nov.	126	—	—	—	—	2*	6	11	24	17	—	—	1	1	4	7	3	4	9	37
18 Dec.	94	—	1	—	—	—	—	8	16	7	—	—	—	1	1	—	3	13	25	19
1948																				
16 Jan.	88	—	1	—	—	—	—	—	7	31	3	—	—	—	—	—	—	1	—	45
11 Feb.	109	—	—	—	—	—	-	—	—	41	24	—	—	—	—	—	—	—	—	44
20 Aug.	105	1	18	38	2	1	—	1	2	—	—	2	29	10	1	—	—	—	—	—
17 Sept.	122	15	40	14	8	—	—	—	—	—	—	—	8	32	5	—	—	—	—	—
8 Oct.	111	—	1	26	22	2	—	18	4	—	—	—	—	2	16	7	4	9	—	—
13 Oct.	120	—	2	30	12	—	—	24	12	—	—	—	1	11	14	—	4	8	2	—
20 Oct.	49	—	—	8	8	—	—	19	—	—	—	—	—	2	5	—	—	5	2	—
29 Oct.	90	—	—	18	20	2	—	13	—	—	—	—	—	4	10	8	10	5	—	—
2 Nov.	128	—	—	4	—	—	—	3	32	40	—	—	—	—	—	—	—	2	5	42
23 Nov.	65	—	—	—	—	—	—	5	18	13	—	—	—	—	—	—	—	—	3	26
24 Dec.	57	—	—	—	—	—	—	—	6	30	—	—	—	—	—	—	—	—	—	21
1949																				
14 Jan.	53	—	—	—	—	—	—	—	6	27	—	—	—	—	—	—	—	—	—	20
4 Feb.	68	—	—	1	—	—	—	—	10	29	2	—	—	—	—	—	—	—	2	24
4 Mar.	65	—	—	—	—	—	—	—	6	41	2	—	—	—	—	—	—	—	—	16
6 Apr.	91	5	6	—	—	—	—	—	—	49	5	2	1	—	—	—	—	—	—	23
26 Apr.	100	4	6	1	—	—	—	—	6	20	17	1	5	3	1	—	—	—	—	36
27 May	57	18	4	—	—	—	—	—	—	11	8	—	1	1	—	—	—	—	—	14
24 June	55	12	5	—	—	—	—	—	—	13	14	3	—	—	—	—	—	—	—	8
9 July	56	10	9	1	—	—	—	—	1	4	3	17	9	—	—	—	—	—	—	2
5 Aug.	69	2	12	12	—	1	—	—	2	—	—	6	20	11	1	—	—	—	2	—
1 Sept.	60	—	1	15	13	3	—	1	—	—	—	—	1	15	7	3	—	1	—	—
22 Sept.	58	—	7	19	8	—	—	—	—	—	—	—	6	9	7	2	—	—	—	—
30 Sept.	62	—	9	6	—	2	—	—	13	—	—	—	2	1	—	—	—	—	26	3
5 Oct.	61	—	2	3	3	1	—	—	24	—	—	—	9	—	2	—	—	4	13	—
19 Nov.	31	—	4	—	—	—	—	—	12	2	—	—	—	—	—	—	—	—	6	7

* Actually stage V+.

TABLE VI. DISTRIBUTION OF GONAD STAGES IN SAMPLES OF *PATELLA VULGATA* FROM MILLPORT

		Male									Neuter	Female								
		Developing					Spawning					Developing					Spawning			
Date	No. in sample	I	II	III	IV	V	IV	III	II	I		I	II	III	IV	V	IV	III	II	I
1946																				
July	104	8	33	17	5	—	—	—	—	—	13	7	16	2	—	—	—	—	—	3
Aug.	115	—	6	31	28	7	—	—	6	1	1	1	14	8	7	3	—	—	2	—
Oct.	92	—	—	—	4	4	—	13	17	—	—	—	—	5	3	1	—	7	31	7
Oct.	109	—	—	—	29	6	1	7	—	—	4	—	—	5	41	13	—	2	—	1
Dec.	128	—	1	2	7	2	—	26	27	8	2	—	—	2	1	—	—	3	21	26
1947																				
Feb.	110	—	2	1	—	—	—	2	17	24	9	—	—	—	—	—	—	2	15	38
May	104	—	8	10	1	—	—	—	3	6	32	—	5	2	—	—	—	—	9	28
Jun.	106	16	14	1	—	—	—	—	1	3	36	2	—	—	—	—	—	—	4	29
July	111	8	29	13	1	—	—	—	—	—	1	23	24	10	1	—	—	—	—	1
Oct.	98	1	25	17	—	—	—	—	—	1	—	1	19	33	1	—	—	—	—	—
Oct.	104	—	—	5	—	—	—	8	24	—	2	—	—	10	7	1	1	19	27	—
Oct.	103	—	—	6	—	—	—	5	22	2	—	—	5	13	4	1	—	12	25	8
Nov.	101	—	—	—	3	—	—	7	24	9	—	—	3	8	4	3	1	8	20	11
Dec.	111	—	—	1	—	—	—	1	11	29	4	—	—	1	1	—	1	1	12	49
1948																				
Feb.	57	—	—	—	—	—	—	—	2	19	12	—	—	—	—	—	—	—	—	24
Mar.	54	—	—	—	—	—	—	—	—	15	18	—	—	1	—	—	—	—	—	20
Apr.	61	1	—	—	—	—	—	—	—	6	45	—	—	—	—	—	—	—	—	9
May	55	4	—	—	—	—	—	—	—	2	45	—	—	—	—	—	—	—	—	4
June	59	11	2	1	—	—	—	—	—	1	27	10	2	—	—	—	—	—	—	5
Aug.	55	—	8	14	2	—	—	—	3	—	—	3	10	9	5	1	—	—	—	—
Sept.	26	—	3	4	1	—	—	—	—	—	—	—	8	7	2	1	—	—	—	—
Sept.	52	—	—	—	—	—	—	5	12	4	—	—	1	5	—	—	—	6	13	6
Nov.	32	—	—	—	—	—	—	—	11	6	—	—	—	—	—	—	1	1	3	10
Dec.	60	—	—	—	—	—	—	1	10	20	—	—	6	—	—	—	—	2	2	19
1949																				
Jan.	60	—	—	—	—	—	—	—	—	16	9	—	—	—	—	—	—	—	—	35
Mar.	55	—	—	—	—	—	—	—	—	13	18	—	—	—	—	—	—	—	—	24
Apr.	54	—	—	—	—	—	—	—	1	13	28	1	—	—	—	—	—	—	—	11
May	51	7	—	—	—	—	—	—	—	6	22	1	—	—	—	—	—	—	—	15
July	50	5	5	1	—	—	—	—	—	5	8	18	6	—	—	—	—	—	—	2
Aug.	54	2	9	2	—	—	—	—	1	—	2	15	20	3	—	—	—	—	—	—
Sept.	53	—	10	5	1	—	—	—	2	1	—	3	26	5	—	—	—	—	—	—
Oct.	61	—	3	1	—	—	—	4	12	5	1	—	4	3	1	—	—	—	11	16
Nov.	29	—	—	—	—	—	—	1	9	1	—	—	5	2	—	—	—	—	4	7
Nov.	31	—	—	—	—	—	—	1	6	5	1	—	2	—	—	—	—	—	2	14

TABLE VII. DISTRIBUTION OF GONAD STAGES IN SAMPLES OF *PATELLA VULGATA* FROM KALLOW POINT, PORT ST MARY, ISLE OF MAN

		Male									Neuter	Female							
		Developing					Spawning					Developing					Spawning		
Date	No. in sample	I	II	III	IV	V	IV	III	II	I		I	II	III	IV	V	IV	III	II
1946																			
9 May	102	—	—	—	—	—	—	—	—	7	81	—	—	—	—	—	—	—	—
15 Aug.	101	10	10	2	—	—	—	—	—	—	60	10	2	—	—	—	—	—	—
21 Aug.	100	11	35	8	1	—	—	—	—	—	14	27	4	—	—	—	—	—	—
3 Oct.	44	—	—	2	12	2	—	1	2	—	1	—	—	2	18	3	—	—	1
17 Oct.	95	—	—	—	6	2	9	22	5	—	—	—	2	7	4	—	—	28	10
1 Nov.	94	—	—	8	8	—	7	20	5	—	1	—	—	12	10	—	—	—	20
25 Nov.	100	—	—	—	—	—	4	14	14	4	—	—	1	1	5	—	—	9	36
13 Dec.	117	1	—	6	—	—	1	29	64	5	—	—	—	—	—	—	—	—	8
1947																			
16 Jan.	106	—	—	—	—	—	—	—	16	1	53	—	—	—	—	—	—	—	—
9 Apr.	112	—	—	—	—	—	—	—	—	9	96	—	—	—	—	—	—	—	—
20 May	115	—	—	—	—	—	—	—	—	9	101	—	—	—	—	—	—	—	—
28 July	97	4	20	10	—	—	—	—	—	—	14	25	24	—	—	—	—	—	—
4 Sept.	108	2	8	23	7	2	—	—	1	—	1	11	38	14	—	—	—	—	1
6 Oct.	110	—	13	27	1	—	—	—	—	—	—	1	30	36	2	—	—	—	—
22 Oct.	121	—	—	11	28	6	—	3	—	—	1	—	—	7	39	20	—	6	—
25 Oct.	103	—	—	7	15	3	—	21	3	—	—	—	1	19	16	3	—	7	8
28 Oct.	111	—	—	3	15	10	13	18	1	—	2	—	—	4	23	6	5	9	2
30 Oct.	107	—	—	1	12	3	5	22	6	—	—	—	1	9	12	7	4	18	7
3 Nov.	110	—	—	2	12	3	6	32	5	—	—	—	—	2	11	4	7	24	2
10 Nov.	103	—	—	—	3	5	4	20	12	5	—	—	—	3	3	4	6	20	8
16 Nov.	105	—	—	—	—	1	3	22	18	4	1	—	—	3	4	4	—	21	14
25 Nov.	105	—	—	—	—	4	1	17	22	1	1	—	1	1	1	6	6	8	22
29 Nov.	101	—	—	—	1	2	5	22	22	4	1	—	—	—	2	3	4	13	15
11 Dec.	55	—	—	—	—	—	1	6	19	1	1	—	—	3	—	—	1	8	9
22 Dec.	50	—	—	—	—	—	—	9	16	1	—	—	—	—	4	2	5	5	7
1948																			
7 Jan.	53	—	—	—	—	—	2	9	12	3	—	—	—	1	—	1	1	8	9
28 Jan.	59	—	2	—	—	—	2	6	10	9	—	—	—	4	2	—	1	5	9
12 Feb.	56	—	—	—	—	—	—	—	—	11	22	—	—	—	—	—	—	—	—
8 Mar.	51	—	—	—	—	—	—	—	—	16	19	—	—	—	—	—	—	—	—
16 Apr.	53	—	—	—	—	—	—	—	—	1	52	—	—	—	—	—	—	—	—
25 May	53	2	—	—	—	—	—	—	—	1	47	—	—	—	—	—	—	—	—
4 July	51	—	—	—	—	—	—	—	—	1	49	1	—	—	—	—	—	—	—
5 Aug.	55	17	2	—	—	—	—	—	—	—	9	27	—	—	—	—	—	—	—
25 Aug.	129	—	24	27	11	—	—	—	—	—	—	5	35	22	5	—	—	—	—
24 Sept.	66	—	9	23	4	—	—	—	—	—	—	—	18	12	—	—	—	—	—
13 Oct.	45	—	—	—	—	—	—	1	5	2	—	—	1	—	—	—	—	1	13
20 Oct.	46	—	—	—	—	—	—	—	8	12	—	—	1	—	—	—	—	—	9
25 Nov.	44	—	—	—	—	—	—	—	1	16	—	—	—	—	—	—	—	—	—
1949																			
27 Jan.	48	—	—	—	—	—	—	—	1	20	5	—	—	—	—	—	—	—	1
18 Apr.	47	—	—	—	—	—	—	—	—	3	40	1	—	—	—	—	—	—	—
25 May	46	10	—	—	—	—	—	—	—	—	22	2	—	—	—	—	—	—	—
11 July	45	11	2	—	—	—	—	—	—	3	21	2	—	—	—	—	—	—	—
9 Aug.	49	11	14	—	—	—	—	—	—	—	4	14	6	—	—	—	—	—	—
5 Sept.	55	—	13	9	—	—	—	—	—	—	—	1	27	5	—	—	—	—	—
17 Oct.	48	—	2	—	—	—	—	—	13	10	1	—	5	—	—	—	—	—	—
23 Oct.	25	—	2	—	—	—	—	—	8	—	—	—	—	—	—	—	—	—	4
24 Nov.	28	—	—	—	—	—	—	—	6	6	—	—	—	—	—	—	—	—	2

TABLE VIII. DISTRIBUTION OF GONAD STAGES IN SAMPLES OF *PATELLA VULGATA* FROM TREVONE

		Male									Neuter	Female								
		Developing					Spawning					Developing					Spawning			
ate	No. in sample	I	II	III	IV	V	IV	III	II	I		I	II	III	IV	V	IV	III	II	I
946																				
Jan.	100	—	—	1	1	—	—	5	20	7	—	—	—	—	—	—	—	4	17	45
Feb.	43	—	—	—	—	—	—	—	5	22	2	—	—	—	—	—	—	—	—	14
June	54	—	—	—	—	—	—	—	13	6	10	—	2	—	—	—	—	—	2	21
July	101	4	1	—	—	—	—	—	—	—	95	1	—	—	—	—	—	—	—	—
Aug.	105	14	41	14	2	—	—	1	—	—	11	10	10	1	—	—	—	—	—	1
Sept.	105	6	22	14	—	—	—	—	—	—	9	16	32	6	—	—	—	—	—	—
Sept.	50	—	2	9	5	1	—	—	—	—	2	1	4	19	7	—	—	—	—	—
Nov.	104	—	—	8	26	8	—	3	—	—	1	—	4	13	23	16	—	1	—	1
Nov.	88	—	2	21	30	3	—	—	—	—	1	—	1	2	6	6	3	11	2	—
Nov.	104	—	2	—	—	—	—	3	32	9	1	1	—	—	—	—	—	—	21	35
Dec.	112	1	5	1	—	—	—	13	64	18	—	—	—	—	—	—	—	—	3	7
947																				
Jan.	102	—	1	—	—	—	—	—	3	50	26	—	—	—	—	—	—	—	—	22
May	105	—	—	—	—	—	—	—	—	—	102	—	—	—	—	—	—	—	—	3
June	98	—	—	—	—	—	—	—	—	—	97	—	—	—	—	—	—	—	—	1
July	102	19	5	—	—	—	—	—	—	13	64	—	—	—	—	—	—	—	—	1
Aug.	104	12	13	—	—	—	—	—	—	—	15	53	11	—	—	—	—	—	—	—
Sept.	103	6	29	5	—	—	—	—	—	—	—	26	28	8	1	—	—	—	—	—
Oct.	104	—	2	29	7	—	—	1	1	1	1	—	7	40	14	1	—	—	—	—
Oct.	99	—	—	4	11	1	1	6	—	1	—	—	—	31	42	2	—	—	—	—
Oct.	103	—	—	2	14	4	—	12	3	—	—	—	3	10	35	11	1	4	4	—
Nov.	102	—	—	—	—	—	5	35	13	1	—	—	—	8	6	2	—	12	18	2
Nov.	104	—	—	—	—	—	—	7	13	12	—	—	2	2	1	5	3	1	11	47
Dec.	104	—	5	1	—	—	1	—	11	25	—	—	—	—	—	—	1	1	24	35
948																				
Feb.	52	—	—	—	—	—	—	—	—	13	17	—	—	—	—	—	—	—	—	22
Mar.	55	—	—	—	—	—	—	—	—	9	26	—	—	—	—	—	—	—	—	20
Apr.	52	—	—	—	—	—	—	—	—	9	34	—	—	—	—	—	—	—	—	9
May	52	2	—	—	—	—	—	—	—	1	45	—	—	—	—	—	—	—	—	4
June	52	1	—	—	—	—	—	—	—	1	40	—	—	—	—	—	—	—	—	10
July	44	3	—	—	—	—	—	—	—	—	35	5	—	—	—	—	—	—	—	1
Aug.	54	11	16	—	—	—	—	—	—	—	—	14	13	—	—	—	—	—	—	—
Oct.	55	—	—	—	—	—	—	1	13	10	—	—	3	1	—	—	—	1	12	14
Nov.	55	—	—	—	—	—	—	—	4	20	—	—	—	—	—	—	—	—	6	25
Dec.	55	—	1	—	—	—	—	—	2	31	—	—	—	—	—	—	—	—	1	20
949																				
Jan.	53	—	—	—	—	—	—	—	—	25	2	—	—	—	—	—	—	—	—	26
Mar.	52	—	—	—	—	—	—	—	—	23	13	—	—	—	—	—	—	—	—	16
Apr.	52	1	—	—	—	—	—	—	—	1	47	1	—	—	—	—	—	—	—	2
May	53	4	—	—	—	—	—	—	—	1	41	1	—	—	—	—	—	—	—	6
July	53	—	—	—	—	—	—	—	—	1	52	—	—	—	—	—	—	—	—	—
Aug.	51	9	1	—	—	—	—	—	—	2	23	13	1	—	—	—	—	—	—	2
Sept.	51	10	9	—	—	—	—	—	—	—	4	26	2	—	—	—	—	—	—	—
Oct.	54	—	6	16	—	—	—	5	4	—	1	—	3	9	5	—	4	1	—	—
Nov.	53	—	1	—	—	—	—	—	17	14	—	—	—	—	—	—	—	—	1	20
Nov.	26	1	—	—	—	—	—	—	—	16	—	—	—	—	—	—	—	—	—	9

TABLE IX. DISTRIBUTION OF GONAD STAGES IN SAMPLES OF *PATELLA VULGATA* FROM PLYMOUTH BREAKWATER

		Male									Neuter	Female							
		Developing					Spawning					Developing					Spawning		
Date	No. in sample	I	II	III	IV	V	IV	III	II	I		I	II	III	IV	V	IV	III	II
1946																			
18 June	141	10	7	2	—	—	—	—	—	15	91	—	—	—	—	—	—	—	1
30 July	137	14	—	—	—	—	—	—	—	—	97	1	—	—	—	—	—	—	—
1 Oct.	112	—	—	9	39	6	—	—	—	—	—	—	2	19	31	6	—	—	—
28 Oct.	120	—	—	10	15	7	—	1	2	—	—	—	1	25	33	14	—	9	3
27 Nov.	134	—	—	3	4	—	1	24	19	1	—	—	1	3	1	—	—	15	54
10 Dec.	141	—	9	4	2	—	—	38	38	5	—	—	1	—	—	—	1	6	29
1947																			
17 Feb.	154	—	12	3	—	—	—	—	31	31	7	—	—	—	—	—	—	1	6
17 May	189	—	—	—	—	—	—	—	—	2	187	-	—	—	—	—	—	—	—
11 July	126	4	1	—	—	—	—	—	—	—	118	—	—	—	—	—	—	—	—
6 Oct.	126	—	1	30	2	1	—	—	—	—	—	—	5	70	15	2	—	—	—
3 Nov.	108	—	2	—	6	1	2	17	12	2	—	—	—	6	16	6	—	9	23
11 Nov.	103	—	—	—	1	2	1	15	13	2	—	—	—	4	8	17	1	13	23
4 Dec.	100	—	4	2	3	7	2	9	4	—	—	—	—	2	—	33	2	10	22
1948																			
16 Feb.	50	—	—	—	—	—	—	—	—	21	5	—	—	—	—	—	—	—	—
2 Mar.	15	—	—	—	—	—	—	—	—	4	1	—	—	—	—	—	—	—	—
5 July	61	5	—	—	—	—	—	—	—	3	37	12	—	—	—	—	—	—	—
7 Aug.	93	17	2	—	—	—	—	—	—	—	14	56	1	—	—	—	—	—	—
1 Sept.	71	4	21	2	—	—	—	—	—	—	—	12	32	—	—	—	—	—	—
14 Oct.	62	—	—	8	10	—	—	3	—	—	—	—	2	15	8	2	4	10	—
2 Nov.	38	—	1	5	—	—	—	—	7	—	—	1	4	10	3	1	—	2	3
17 Nov.	100	—	2	3	1	—	—	5	24	7	—	—	5	12	7	—	—	10	5
30 Nov.	99	—	—	4	—	—	—	6	21	3	—	—	8	10	4	1	1	13	15
21 Dec.	85	—	—	—	—	—	—	—	2	38	—	—	—	—	—	—	—	—	—
1949																			
1 Feb.	75	—	—	—	—	—	—	—	—	29	—	—	—	—	—	—	—	—	—
2 Mar.	80	—	—	—	—	—	—	—	—	27	16	—	—	—	—	—	—	—	—
3 May	82	2	—	—	—	—	—	—	1	6	61	4	3	—	—	—	—	—	—
28 June	50	1	—	—	—	—	—	—	—	1	47	1	—	—	—	—	—	—	—
9 Aug.	52	4	3	—	—	—	—	—	—	—	30	12	—	—	—	—	—	—	—
10 Sept.	66	9	18	—	—	—	—	—	—	—	—	25	13	—	—	—	—	—	—
12 Oct.	56	—	4	14	2	—	—	—	—	—	—	—	11	24	1	—	—	—	—
27 Oct.	82	—	1	—	—	—	—	—	29	1	—	—	—	—	—	—	—	—	15
23 Nov.	40	2	—	—	—	—	—	—	6	10	—	—	—	—	—	—	—	—	1

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THE ABNORMAL DEVELOPMENT OF PLAICE EMBRYOS AND LARVAE IN MARINE AQUARIA

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(Plate I and Text-figs. 1–15)

We know that a mature female plaice can liberate many thousands of eggs into the sea during the spring spawning season in the southern North Sea (Simpson, 1951). We also know that a very small percentage indeed of these eggs develops into fish of marketable size. At all stages in its life history a plaice is confronted with environmental hazards, which, if not overcome, may have fatal consequences.

The pelagic egg and subsequent larval phase of marine teleosts appear to be particularly vulnerable to adverse conditions in the sea. Rollefsen (1930, 1932) demonstrated the lethal effect of agitation on early cod eggs and discussed wave-action as a mortality factor. Battle (1944) elaborated this work for other teleost species. Tester (1942) observed a high mortality of herring eggs spawned inshore, on weeds, along the south-east coast of Vancouver Island, and thought this loss was due to respiratory difficulties resulting from low carbon dioxide or high oxygen content of the water, a theory not substantiated by the work of Kelley (1946). There must also be considerable losses of eggs and larvae due to the activity of predators. Spent herring caught in the Southern Bight in early spring are frequently found with stomachs full of plaice eggs.

Undoubtedly, estimates of early mortality in the sea are needed for a better understanding of the population dynamics of a particular fishery. At the same time, rearing experiments in the laboratory, to determine the effect of known environmental changes on the development and survival of pelagic eggs and larvae, should give some knowledge of the possible causes of natural death.

Salt-water fish are notoriously difficult to raise. Petersen (1894), after several abortive attempts to rear plaice from artificially fertilized eggs, was emboldened to say, '...at the pelagic stage, after the yolk has been absorbed, it (the plaice post larva) cannot be kept alive in aquaria; at this stage the larva must be caught in open water and this is not always easy'. Cunningham (1894), using circulating water at 12° C in his aquaria, also failed to rear whiting, flounder and plaice eggs to the post-larval stage. Dannevig (1897) succeeded in taking a small proportion of an artificially fertilized batch of plaice eggs through and beyond metamorphosis for the first recorded time.

Since these early attempts, Norwegian workers have had more success with plaice, cod and herring, accounts of which have been given by Rollefsen (1940) and Dannevig & Hansen (1952).

At Lowestoft, during our recent experiments on plaice reared under static sea-water conditions in temperature-controlled aquaria, we encountered the usual difficulties. However, by the close observation of living material under known laboratory conditions, it becomes possible, first of all, to recognize normal healthiness, and then, against this standard, to detect even slight symptoms of abnormality. Knowledge of these symptoms and their causes aids the development of efficient rearing techniques and the interpretation of experimental results.

Some abnormalities of fish larvae in marine aquaria are already known. Dannevig (1897) observed that larvae from newly hatched plaice eggs could readily be divided into two fractions: a minority of healthy, richly pigmented individuals found ranging for food at all levels in the aquarium, and a majority of abnormal specimens which, after early hyperactivity at the surface, soon lost their pigment, and during long quiescent periods were to be found suspended head downwards near the bottom of the jar. Gorham's early work on the gas-bubble disease of adult marine fish (1901) has been followed by observations on the same complaint in larvae with swim-bladders, by Kotthaus (1939), Soleim (1940) and Henly (1952). Plaice larvae are not affected by this disease. Bückmann, Harder & Hempel (1953) found that herring larvae in lighted glass-vessels often sustain deformations of the head by continual collision with the walls of the jars. Attacks by *Vibrio anguillarum* and *Lentospora cerebralis* (Dannevig & Hansen, 1952), and by bacteria, can produce abnormality and death in marine aquaria.

Trout larvae in freshwater aquaria frequently suffer from a fluid inflammation of the yolk-sac, known generally as *Hydrocoele embryonalis*, or as 'Dotterblasenwassersucht' by German workers. Dieterich (1938) proved the offspring of young parents to be more susceptible to 'yolk-sac dropsy' than the issue of older fish, and that offspring of crosses of related species are similarly prone.

The present paper describes a further common, abnormal condition in plaice, and attempts to explain it in terms of what is already known of the structure and physiology of pelagic marine larvae.

Collection of Material and Rearing Methods

Plaice eggs were periodically collected by stramin net in the Southern Bight and off Flamborough, during the spawning season of 1953. Additional supplies of eggs were available from an artificial fertilization carried out in the Irish Sea area. After separation from the rest of the catch, plaice eggs at various stages of development were kept in 7 lb. jars at 6° C with frequent water changes. On arrival in the laboratory, the eggs were transferred to fresh sea water from the spawning area in glass vessels cooled to 6° C ±0·5° in a large tank. 150–200 eggs were pipetted into each vessel of 1500 ml. capacity. The eggs were gently aerated up to the start of hatching; the emergent larvae were then removed into identical vessels, in batches of fifty. *Artemia salina* nauplii were introduced as food into the aquaria 4 days after hatching.

The shortcomings of this technique are manifold. Temperature alone was adequately controlled. In such small volumes of static sea water there must be inevitable ionic variations with the excretion and accumulation of carbon dioxide and other metabolites. Plaice larvae are able to adapt themselves to these gradual changes, some individuals more completely than others. A gross water renewal invariably kills off the weaker brethren. Ionic changes are also brought about on the addition of living food to the jars, and by the use of air from which the carbon dioxide has been incompletely removed.

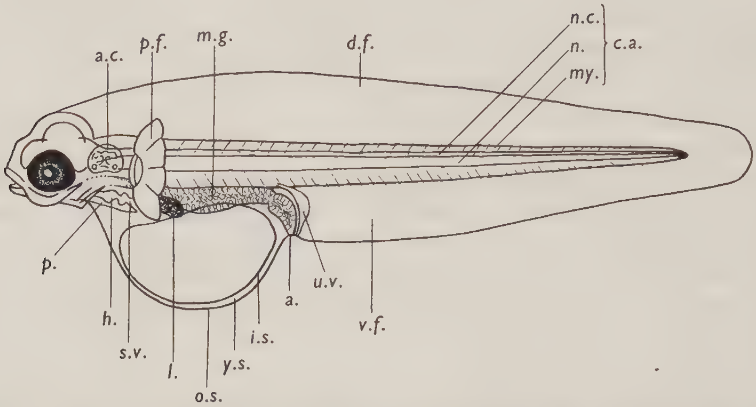
Light conditions were uncontrolled; noticeable changes in larval activity were caused by the use of inspection lamps, thus disturbing the natural diurnal cycle of activity as witnessed by their feeding habits (Shelbourne, 1953).

Where a supply of continually renewed and chemically constant sea water is available, many of the obstacles to the successful rearing of marine teleost eggs and larvae are removed. However, it must be recorded that during the 1954 plaice-spawning season, batches of artificially fertilized eggs were taken through to a normal early post-larval stage with no water change and no aeration, under constant temperature and light conditions. On the introduction of washed *Artemia* nauplii into the jars, the majority of the larvae started feeding. Successive additions of food called for frequent water changes; it was under these rapidly changing physico-chemical conditions that the larvae began to sicken.

The Features of a Normal Larva and the Course of its Yolk Loss

Text-fig. 1 is a diagram of a normal newly hatched plaice larva 6·5 mm long. The fore-, mid- and hind-brains are clearly seen through the transparent cartilage of the cranium, whilst the heavily pigmented eyes and expanded auditory capsules are the most conspicuous of the associated sense organs. The nervous tissue of the brain narrows into the nerve cord posteriorly, which, together with the notochord, developing median blood vessels, and investing muscle myotomes, forms the larval axis or trunk.

The angular mandible is normally mobile soon after hatching, its articulation with the upper jaw producing a mouth gape sufficiently wide to accommodate the large, thin-walled diatoms and small zooplankton of northern waters. The future branchial apparatus is as yet incomplete; the gill clefts are open, but the gill bars lack branchial filaments. Even at this early stage, the simple gut tube is already coiling to the right, and is wedged between the ventral surface of the larval axis and the dorsal side of the underslung yolk-sac. Sphincters divide the alimentary canal into fore-, mid- and hind-gut, opening ventrally at the anus. The liver is a conspicuous gut appendage lying just behind the heart.



Text-fig. 1. Early plaice larva: side view. *a*, anus; *a.c.*, auditory capsule; *d.f.*, dorsal marginal fin; *h.*, heart; *i.s.*, inner yolk-sac; *l.*, liver; *m.g.*, mid-gut; *my.*, myotome; *n.*, notochord; *n.c.*, nerve cord; *o.s.*, outer yolk-sac; *p.*, pericardium; *p.f.*, pectoral fin; *s.v.*, sinus venosus; *u.v.*, urinary vesicle; *v.f.*, ventral marginal fin; *y.s.*, yolk-sac sinus.

In the ventro-lateral tissue of the trunk, above the gut, run the two urinary ducts, each of which connects in front with a pronephros behind the auditory capsules. The two ducts coalesce posteriorly as the urinary vesicle, opening behind the anus.

The heart, in its pericardium, lies underneath the oesophagus, and at hatching is differentiated into a muscular ventricle, an auricle, and a membranous sinus venosus. Anteriorly, the ventricle is twisted upwards and forwards to the ventral aorta, whilst posteriorly the sinus venosus opens directly into the yolk-sac sinus, a space between the membrane of the outer yolk-sac and the periblast layer, or inner sac surrounding the fluid yolk. There are neither cellular structures nor oil globules in the yolk, and it has no direct communication with the gut. The outer yolk-sac is but a ventral extension of the very thin integument completely clothing the larva, except at the gut and renal openings. This membrane forms the large marginal fin extending from the head above the larval axis, round the caudal extremity and back ventrally as far as the anal aperture. The two lamellae of the continuous fin are separated from one another, and supported by turgor in the serous subdermal space between (Text-fig. 4). The paired pectoral fins project outwards and backwards, just behind the auditory vesicles.

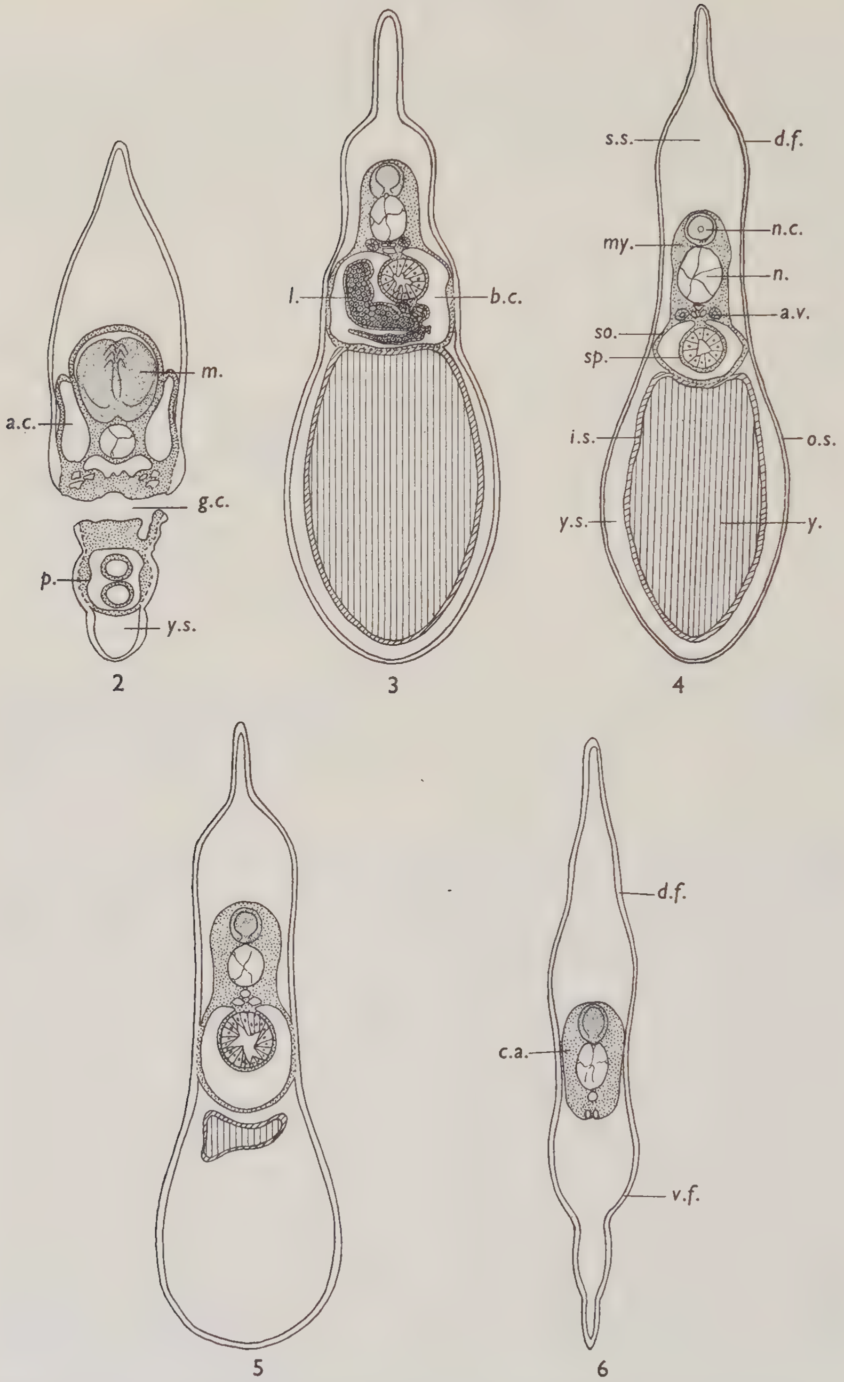
A normal plaice is conspicuously pigmented at hatching, with yellow xanthophores and black melanophores, which have a stellate or corpuscular form, according to their degree of contraction. They are generally confined, in early larval life, to the superficial tissues of the main larval axis, though in good environmental conditions, xanthophores either develop on, or migrate into, the marginal fin above the caudal axis. Only black melanophores are found on the inner yolk-sac; these are sometimes also attached to the inner face of the outer sac, thus traversing the yolk-sac sinus.

Finer structural details are shown in Text-figs. 2–6, which are diagrammatic reconstructions of transverse sections through the yolk-sac and caudal regions of a normal newly hatched plaice larva.

The larval trunk (Text-fig. 4) consists of nerve cord, notochord, blood vessels and urinary ducts, all invested by the mesodermal musculature of the myotomes. During embryonic devlopment, a longitudinal fold has grown down each side from the axial mesoderm. The proximal edge of each fold, the splanchnopleure, contributes a lining of coelomic epithelium to the outer surface of the gut and its derivatives, by median fusion. The distal edges, or somatopleure, are reflected outwards to the integument, then inwards to join under the gut, and will eventually give rise to the ventro-lateral body wall of the post-larva, when the yolk has finally disappeared. At this early stage, the somatopleure is indifferently attached to the integument above the inner yolk-sac, whilst the ventral extension under the gut is thin and attenuated, and in some sections difficult to distinguish from the periblast tissue on the dorsal side of the inner yolk-sac. Its continuity ventrally is best seen in sections of the posterior gut region (Text-fig. 5).

The inner yolk-sac, or periblast of early workers, is a thick syncytial membrane (Meek, 1913) surrounding the fluid yolk. It is formed by the activity of the ventral blastopore lip during gastrulation, and now lies inside the outer ectodermal yolk-sac, separated from it ventrally and laterally by a serous space, the yolk-sac sinus. This sinus is formed by the early breakdown into mesenchyme, of the extra-embryonal mesoderm layer laid down between the two sacs during gastrulation (Meek, 1924). The integument of the outer yolk-sac is continuous with that of the marginal fin, and the yolk-sac sinus has lateral connexions with the lumen of the marginal fin due to the indifferent attachment of the somatopleure to the integument at gut level. The continuity of the yolk and fin spaces will be discussed in more detail later.

The transparent skin is very thin, and according to McIntosh & Prince (1890) consists of an external layer of flattened, corneal cells and an inner Malpighian layer with a very loose cell texture. Meek (1924), on the other hand, states that in the cod the integument has a syncytial nature, reinforced by mesodermal elements. According to which view is adopted, the serous space between the integument and larval axis dorsally and the inner yolk-sac



Text-figs. 2–6. Diagrams of transverse sections through an early plaice larva: through (2) pericardial region, (3) anterior part of yolk-sac, (4) mid-yolk-sac region, (5) posterior part of yolk-sac, and (6) caudal region. *a.c.*, auditory capsule; *a.v.*, axial vessels; *b.c.*, body cavity; *c.a.*, caudal axis; *d.f.*, dorsal marginal fin; *g.c.*, gill cleft; *i.s.*, inner yolk-sac; *l.*, liver; *m.*, medulla; *my.*, myotome; *n.*, notochord; *n.c.*, nerve cord; *o.s.*, outer yolk-sac; *p.*, pericardium; *so.*, somatopleure; *sp.*, splanchnopleure; *s.s.*, subdermal space; *v.f.*, ventral marginal fin; *y.*, fluid yolk; *y.s.*, yolk-sac sinus.

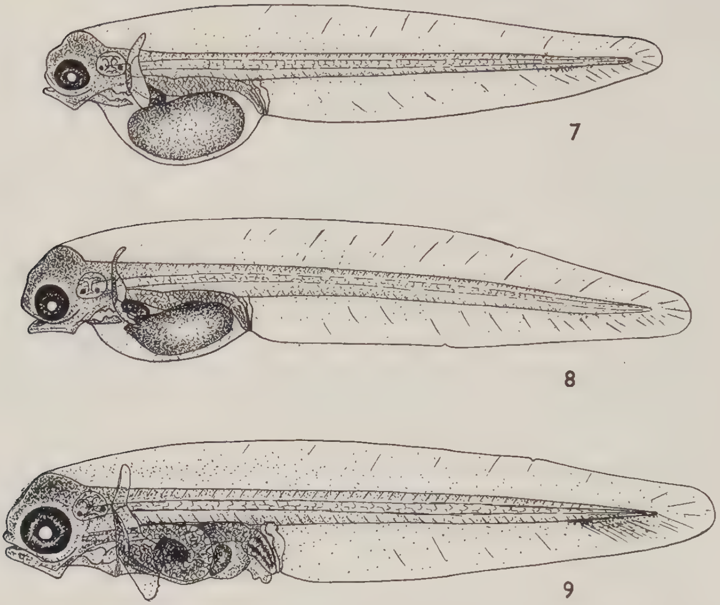
ventrally is either a subepidermal or a subdermal space. The latter term will be used in this paper.

Text-fig. 3 is a transverse section through the liver. The lateral, longitudinal fusion of the somatopleure to the integument is still apparent, but just in front of the liver, where the somatopleure expands into the pectoral mesoderm, the attachment is considerably weakened (Pl. I, fig. 2). Further forward still (Text-fig. 2), the segmented trunk mesoderm merges with the plate-like mesoderm and loose mesenchyme of the head, which is giving rise to skeletal structures, blood vessels and musculature. The head integument is closely integrated with the underlying tissue in front of the optic lobes of the brain; for the most part, the brain, eye and ear capsules lie in a fluid-filled space, continuous with that of the marginal fin. An embryonic downgrowth of two mesoderm folds under the head has produced the pericardium and heart by ventral fusion; at hatching, the wall of the pericardium is still largely separated from the skin beneath, to give the forward tip of the yolk-sac sinus.

In the caudal region (Text-fig. 6), behind the yolk, the close attachment of the integument to the sides of the somites of the larval axis divides the subdermal space into the lumina of the upper and lower portions of the marginal fin.

To sum up, a young normal plaice larva is clothed by a thin integument, which is strongly attached to the mesoderm in the anterior head region and to the sides of the caudal axis; it is indifferently attached above the yolk-sac sinus, particularly in the region of the pectoral fin. The inner yolk-sac is thus a bag of yolk lying in a ventral extension of this skin, its subdermal space (yolk-sac sinus) incompletely roofed over by somatopleural fusion with the integument. It is cut off behind by mes-ectodermal fusion round the rectum and urinary vesicle, and in front by the integration of the integument with the mesoderm on the underside of the head.

Although the normal embryo is coiled inside an egg-shell seldom greater than 2 mm in diameter, the freed larva may be 6 mm long, with straight axial structures and a turgid marginal fin. Shortly before hatching a marked diminution occurs in the volume of the yolk-sac system. The larva emerges with a boat-shaped yolk which continues to diminish smoothly and evenly for 8–10 days at 7° C (Text-figs. 7–9). Towards the end of larval life the small inner yolk-sac becomes pushed up into the arch formed by the looping intestine, so that the ventral surface of the animal, under the gut, is uninterrupted by pendulous projections of any kind. A small spherule of inner yolk-sac tissue may persist in what is now the posterior part of the body cavity for some time after the main mass of fluid yolk is lost. The final disappearance of this yolk vestige marks the end of the arbitrary larval stage and the beginning of the post-larval life of the plaice.



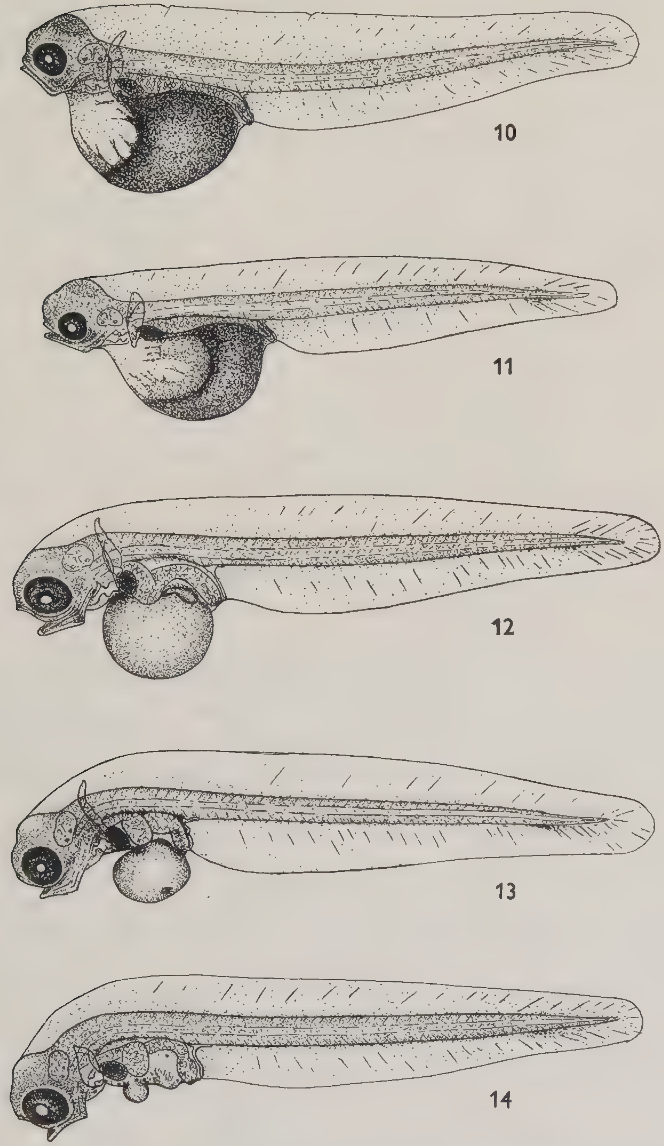
Text-figs. 7–9. Plaice larvae at (7) 24 h, (8) 3–4 days, and (9) 2–3 weeks, showing the normal course of yolk loss during development. Drawings are exact reproductions of preserved larvae. × 13.

ABNORMAL YOLK LOSS AND THE DEVELOPMENT OF AXIAL BENDING

In contrast to the normal course of observable yolk loss just described, tank-reared larvae are prone to abnormal and uneven yolk resorption. Towards the end of the larval phase, affected plaice invariably develop a marked dorso-ventral bending of the axial structures above the gut.

Abnormal individuals hatch with an unusually large inner yolk-sac, excavated anteriorly where the sinus venosus opens into the yolk-sac sinus (Text-fig. 10). This anterior dimpling of the yolk is very characteristic and is caused by an accumulation of plasma in the yolk-sac sinus underneath the pericardium. The larval axis may still show signs of embryonic curling as the fish lies at the surface, moving only when touched.

The subpericardial space enlarges as the inner yolk-sac decreases, so that the outer investing sac remains swollen. At the same time, the periblast tissue of the inner sac adheres closely to the inside surface of the outer sac, forming fingers of tissue extending forwards towards the heart (Text-fig. 11). The dimpling of the yolk is usually intensified as the surface area of the outer sac slowly decreases. The yolk mass comes to occupy the posterior part of the outer sac, which, when viewed from above, appears much more bulbous than



Text-figs. 10–14. Plaice larvae showing abnormal course of yolk loss and the gradual bending of the axis above the gut during development: (10) 12 h, (11) 24 h, (12) 8 days, (13) 2 weeks, and (14) 3 weeks. Exact reproductions of preserved larvae. × 13.

the streamlined sac of a normal specimen. The larva is now active at or near the surface of the aquarium. In spite of abnormal yolk loss, the larva appears to be healthy and its axial structures straight. At a slightly later stage in yolk retraction, the cup-shaped anterior excavation becomes obliquely inclined, so that the direction of inner sac regression is now postero-dorsally, instead of posteriorly. At its upper edge, the inner sac remains in close contact with the visceral mass above.

Normally, that portion of the outer sac membrane underlying the heart progressively approaches and knits from before backwards with the pericardium, whilst the remaining yolk in its periblast retracts up into the arch formed by the coiling intestine. In abnormal specimens with a similar degree of yolk loss (Text-fig. 12) some fusion takes place under the heart, but it is limited by the accumulation of plasma in the original subpericardial space, which has now extended so as to occupy almost the whole of the outer yolk-sac. This space is in reality a swollen yolk-sac sinus. The anterior edge of the outer sac is characteristically reflected forwards from its point of attachment to the pericardium; the sac is now a turgid, subspherical, pendulous structure underneath the gut.

It is at this stage of abnormal yolk loss that the axial structures above the coiled gut begin to bend dorso-ventrally. The mouth starts to gape, and the arch of the intestine becomes more pronounced as the head and anus approach one another. The larva is hyperactive, swimming jerkily in a plane obliquely inclined to the surface. This behaviour contrasts with that of a normal larva of the same age, which cruises slowly round the depths of a jar with the minimum of effort, only interrupting its progress to investigate and perhaps catch food particles of a suitable size. During frequent quiescent periods, abnormal larvae lie upside down at the surface, supported by the swollen and buoyant outer yolk-sac.

Text-fig. 13 shows a larva in an advanced pathological state. The inner yolk-sac has been reduced to a band of tissue underneath the arching gut. The still turgid outer sac has been considerably reduced, whilst its subspherical proportions are further accentuated by increased fusion with the tissue beneath. The heart itself is noticeably congested in living specimens. Bending has intensified so as to incline the plane of the pectoral fin to the vertical, and the mouth is fixed in a permanent gape by tension on the mandibular musculature. The caudal axis is thin and compressed, the myotomes losing their distinctness. By now, the larva has lost its buoyancy and is to be found feebly spiralling near the bottom of the jar, showing no interest in offered food particles. In contrast to normal larvae, pigmentation is poor; some black chromatophores may persist, but all the yellow xanthophores have disappeared.

Finally, the outer yolk-sac becomes just visible as a slight ventral protuberance, and the head is bent downwards almost at right angles to the caudal axis (Text-fig. 14). The inner yolk-sac has disappeared, and the larva lies intermittently vibrating on the bottom of the jar. Dehydration soon follows, marked by the spread of opacity from the anterior and posterior extremities of the animal.

All abnormal larvae reared in artificial conditions do not display the same severe axial bending as the series just described. Generally speaking, the earlier the onset of abnormal symptoms, the greater is the distortion at the end of the larval phase, and the smaller are the chances of survival through to the post-larval stage.

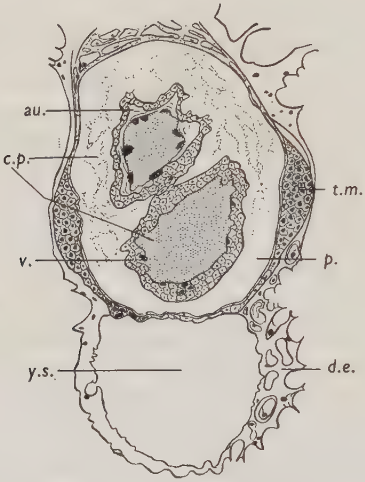
Some idea of the high incidence of this complaint is given by experimental data for 1953. In January, 74% of North Sea plaice eggs used in the laboratory gave rise to severely bent larvae. In February, 66% were abnormal and only 5% of the remainder survived through to the post-larval stage. Bending also occurred in April on a smaller scale. Preserved specimens from earlier years frequently show signs of abnormality. Nor is this complaint confined to plaice-rearing work at Lowestoft. Fullarton (1891) made a series of drawings of whole larvae, as part of his paper on the morphology of plaice development; the younger larvae show signs of abnormal yolk loss, whilst the oldest is very definitely bent.

The Mechanics and Cause of Axial Bending

The course of abnormal and normal yolk loss may now be reconsidered against the structural background. The first characteristic sign of abnormality is the accumulation of fluid in the anterior tip of the yolk-sac sinus, to form the 'subpericardial space', a structure erroneously considered normal by McIntosh & Prince (1890) for the plaice, and by Ryder (1883) for the embryo shad. The space is significantly spherical in shape; it is contained partly by the outer yolk-sac and pericardial wall, and partly by the dimpled membrane of the inner yolk-sac, the tissue of which is left sticking to the inside surface of the outer sac during yolk loss. These indications suggest that the contents of the outer yolk-sac are under a slight pressure. Two conditions are necessary for the development and maintenance of this pressure: first, an increase in the tension of the outer-sac membrane, and secondly, the outer yolk-sac must be a closed system.

During the normal course of larval yolk loss, the surface areas of the inner and outer yolk-sacs rapidly diminish in phase with one another, until finally the inner sac completely disappears and the outer sac becomes the integument covering the thickening mesoderm under the gut. Abnormally, on the other hand, the speed of diminution of the outer sac is much less than that of the inner sac, due to the persisting accumulation of plasma in the sinus between the sacs. Although the outer sac membrane may have elastic properties, there is evidence that it loses surface area by an active process of degeneration and reorganization, rather than one of mechanical reassertion.

Text-fig. 15 is a transverse section of a newly hatched larva, through the front part of the outer sac underneath the heart, showing this active process at work. Before degeneration sets in, the thin membrane is only sparsely sprinkled with nuclei. As degeneration proceeds, the affected area becomes much more richly supplied with vesicles and nucleated structures, some of which may be budded off externally, resembling the process of inner-sac corpusculation described by Ryder (1883) and Williamson (1898). Others open, and presumably discharge their contents at the outer face of the integument, leaving sharply defined depressions in the matrix of the membrane, which is thickened and puckered at this point. These activities can be followed in the living larva.



Text-fig. 15. Transverse section through the pericardium of a young plaice larva, × 150. *au.*, auricle; *c.p.*, coagulated plasma; *d.e.*, degenerating ectoderm; *p.*, *pericardium*; *t.m.*, thickening mesoderm; *v.*, ventricle; *y.s.*, yolk-sac sinus.

Despite corpuscular discharge, the membrane remains imperforate. It is significant that integumental activity occurs for the most part anteriorly, and in close proximity to growing mesoderm. As a result, the two tissues knit closely together from before backwards, the surface area being reduced in the process. The early direction of mes-ectodermal fusion is shown by the receding anterior edge of the outer sac in Pl. I, figs. 1–4; only at a late larval stage does similar activity start in front of the anus, nipping in the posterior edge of the sac. The somatopleural walls each side of the body cavity behind the heart become thickened, and, together with the ectodermal contribution from the degenerating outer yolk-sac, form the ventral body wall of the larva.

When yolk loss is normal, the tension set up in the outer sac, by its fusion with mesoderm under and behind the heart, pushes the remaining yolk in its periblast up into the arch of the intestine. Abnormal yolk loss does not prevent, but hinders, the trend towards degeneration of the outer sac membrane as it knits with the underlying mesoderm anteriorly; the tension thus generated fulfils the first condition needed to produce a slight pressure in this sac.

The second condition is that the outer sac remain a closed system. It was first shown by Fulton (1898) that a large volume of low density yolk diluent enters the egg at maturation in the ovary, thereby enabling it to float in sea water when liberated. It is clear that the overall volume enclosed by the embryonal integument can only decrease, during incubation, within the limit laid down by the initial reserve buoyancy, if the egg is to remain floating. As soluble nutrients are withdrawn from the dilute yolk during tissue-synthesis and respiration, so the yolk diluent accumulates beneath the integument and separates it from the underlying mesoderm to form the system of subdermal spaces so characteristic of pelagic embryos and larvae. In this way, the embryonal volume is maintained; but equally important is the fact that, as a result of integumental separation, the yolk-sac sinus is put in lateral communication with the lumen of the dorsal marginal fin, so that yolk derivatives have easy access to the growing tissues, without the intervention of the heart and ill-developed vascular system (Shelbourne, 1955).

In abnormal plaice larvae the lateral subintegumental route connecting the yolk sinus with the fin space either fails to develop or becomes occluded. The somatopleure fold, by its non-separation from the integument at gut level and in the region of the pectoral fin, cuts off these two subdermal spaces. As the vascular circuit outside the yolk-sac sinus is simply a by-pass with neglible volume at this early larval stage, the outer sac is virtually a closed system, conforming to the second abnormal condition necessary for the maintenance of a slight internal pressure.

The inner yolk-sac is indistinctly separated from the body cavity by the weak ventral extension of the somatopleure under the gut. During abnormal yolk loss this thin membrane ruptures; the body cavity becomes continuous with the swollen sac underneath and is likewise inflated. The sac and body cavity system then has an hour-glass shape, the waist being an inpushing ring of fusing integument and mesoderm. As this ring closes under slight pressure, so the axial structures above the gut bend in conformity. Further mes-ectodermal fusion under the gut, to complete the ventral body wall, takes place with the axis thus bent, with the result that, when the pendulous sac disappears in anything up to a month, the distortion is maintained.

The cod also has a pelagic embryo, but it is not prone to the distortion already described for the plaice. Pl. I, fig. 1, is a slightly oblique transverse section through the pectoral fin region of a young cod larva, to show the great extent of the subdermal space and the complete separation of the integument from the underlying structures. This separation is visible in the living larva towards the end of the yolk-sac stage. The yolk sinus is continuous with the lumen of the marginal fin, to give free access to yolk derivatives to all parts of the larva. The cod may be said to be a classical example of the pelagic mode of teleost egg development.

During normal plaice development such gross separation of integument from underlying tissue does not occur; turgor is never as great, and its degree is much more critical. In the static water conditions of our rearing experiments, it is likely that chemical deterioration of the water, with the accumulation of respiratory metabolites, particularly carbon dioxide (Burfield, 1928), can impair the efficiency and impermeability of the embryonic and larval integument. Water will then be lost and salts gained, under the osmotic gradient, and with the reduction in turgor the integument will come to lie closer to the underlying mesoderm. If turgor is sufficiently reduced by poor conditions at an early embryonic stage, the important connexion between the yolk and fin spaces may never develop. Moreover, with loss of water there will be an increase in salt concentration. The internal salt balance must be restored, or the biochemical processes of normal development will be seriously affected. No mechanism of salt regulation for the marine fish embryo is yet known; if salt tolerance is slight, then it is easy to understand why marine larvae are difficult to rear in shore aquaria.

Significant metabolite accumulations are not likely to occur in the sea during the early life of the plaice, since chemical conditions are practically constant. One might therefore expect to find but a slight incidence of axial bending in larvae from plankton hauls. Only a single suspect has been found during the investigation of some thousands of sea larvae, although an adult plaice with a badly bent axis was landed on Lowestoft fish market in the spring of 1954.

My thanks are due to Mr G. T. Thacker of this laboratory, for executing the drawings in this paper; and also to Dr H. A. Cole of the Fisheries Laboratory, Burnham-on-Crouch, and to Mr A. C. Simpson, of the Shellfish Experimental Station, Conway, for their helpful criticisms of the original manuscript.

SUMMARY

In 1953 laboratory attempts to rear plaice post-larvae were marred by the regular occurrence of structural distortion in late larval life. Normally the yolk-sac system diminishes rapidly and evenly during the short larval phase; abnormally the outer integument of the yolk-sac houses a persistent accumulation of plasma.

Under normal circumstances the yolk-sac sinus communicates laterally with the subdermal space of the dorsal marginal fin, providing free access of fluid yolk derivatives to the growing tissues. Abnormally the lateral channels become occluded or fail to develop, and yolk derivatives, under a slight

pressure, are trapped in the outer sac. This prevents the smooth fusion of mesoderm and ectoderm to form the ventral part of the body wall, and ultimately causes structural distortion of the larval axis above the body cavity.

Cod larvae never suffer this distortion, because the turgor within their subdermal spaces is sufficient to separate completely the integument from the somitic mesoderm above the yolk sac. The degree of turgor in plaice embryos and larvae is much more critical, and water loss under the osmotic gradient, in poor tank conditions, may account for the described abnormality.

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EXPLANATION OF PLATE I

⊢⊣, indicates progress of mes-ectodermal fusion, from before backwards.

Fig. 1. Transverse section through the pectoral region of a cod larva; stained haematoxylin.

Fig. 2. Transverse section through the pectoral region of a plaice larva: stained haematoxylin.

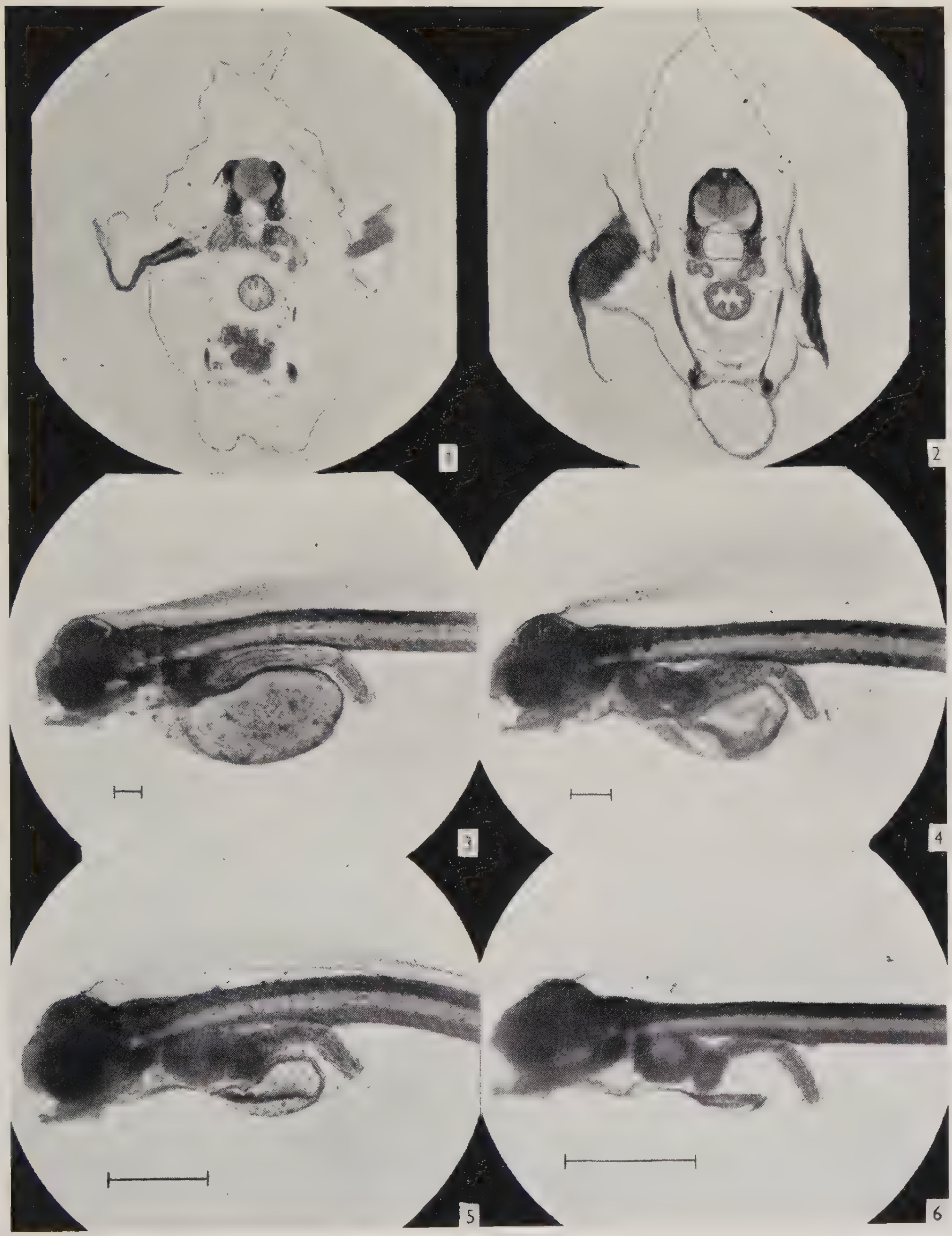
Fig. 3. Preserved plaice larva; 1 day old. Early mes-ectodermal fusion anteriorly, under the pericardium.

Fig. 4. Three days old. Mes-ectodermal fusion now extends posteriorly beyond the pericardium.

Fig. 5. Four days old. Inner and outer yolk sacs much reduced. Integument knitted to underlying mesoderm beyond the level of the liver.

Fig. 6. Nine days old. Mes-ectodermal fusion almost complete ventrally.

Figs. 1–2, ×68.
Figs. 3–6, ×23.



Figs. 1–6.

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NEUROHORMONES OF INVERTEBRATES

I. CARDIO-REGULATORS OF *CYPRINA* AND *BUCCINUM*

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(Text-figs. 1–5)

The present study had two aims: first to seek among the molluscs of the Plymouth region, one with a heart suitable for the bioassay of acetylcholine and 5-hydroxytryptamine; and secondly, to examine molluscan nervous tissue for the presence of cardio-regulator substances. Brief reports of some of the results have appeared elsewhere (Welsh, 1953*a*, 1954*a*).

Material and Methods

Following a preliminary study of the hearts of *Pecten maximus* (L.), *Modiolus modiolus* (L.), *Mytilus edulis* L., *Cyprina islandica* (L.), and *Buccinum undatum* L., the last two were considered most promising for purposes of bioassay. The common whelk, *Buccinum*, was available in adequate numbers but the supply of the bivalve, *Cyprina*, was limited.

The *Cyprina* heart was isolated and perfused in the same manner as previously described for the heart of *Venus mercenaria* (Welsh & Taub, 1948). The *Buccinum* heart was exposed and a glass cannula inserted into the ventricle either by way of the auricle or the aorta. When the cannula was inserted via the auricle, two or three separate ligatures of rather coarse thread were used to secure the cannula. This usually prevented the tearing of the delicate auricle from the thick-walled ventricle. The *Buccinum* ventricle was perfused in a manner similar to that used by Alexandrowicz & Carlisle (1953) in perfusing hearts of decapod crustaceans. Sea water was the perfusion fluid.

When examining a tissue for the presence of acetylcholine (or acetylcholine-like substance) an extract was made by grinding with sand in the presence of eserine, 1:10,000, or grinding after heating the tissue at 100° C for a few minutes. When looking for an opposing, heart-exciting substance, a tissue was ground and allowed to stand at room temperature for 30 min before filtering, or, the extract that had been made with eserinized sea water was brought to pH 9–10 with NaOH and boiled for 10 min to hydrolyse the acetylcholine.

Several drugs were used to help in the identification of the suspected cardio-regulator substances. Among them were lysergic acid diethylamide or LSD (supplied by Sandoz Products Ltd., London), 5-hydroxytryptamine (supplied as serotonin creatinine sulphate by the Abbott Laboratories), and 2:5 bis-(3-diethylaminopropylamino)-benzoquinone bis-benzylchloride or mytolon (supplied by Sterling-Winthrop Research Institute).

Concentrations of drugs are expressed in grams per millilitre of fluid surrounding the heart (*Cyprina*) or passing through the heart (*Buccinum*); thus: 10^{-9} ACh $= 10^{-9}$ g acetylcholine chloride per millilitre of perfusing fluid or 0·001 μg/ml.

RESULTS

The Cyprina *heart*

This heart was tested for its usefulness in the bioassay of acetylcholine and 5-hydroxytryptamine. The threshold to acetylcholine lay between 10^{-10} and 10^{-9} g/ml. (see Fig. 1). Unlike the *Venus* heart, which usually shows a graded slowing and a graded decrease in amplitude of the beat with gradually increasing concentrations of acetylcholine, the *Cyprina* hearts which were tested showed only a slowing. One heart with a normal frequency of 12 beats/min was unaffected by 10^{-10} acetylcholine; at 10^{-9} acetylcholine the frequency was 9·5 beats/min; at $2{\cdot}5 \times 10^{-9}$ acetylcholine, 8 beats/min; at 5×10^{-9} acetylcholine, 6 beats/min; while at 10^{-8} acetylcholine the heart stopped.

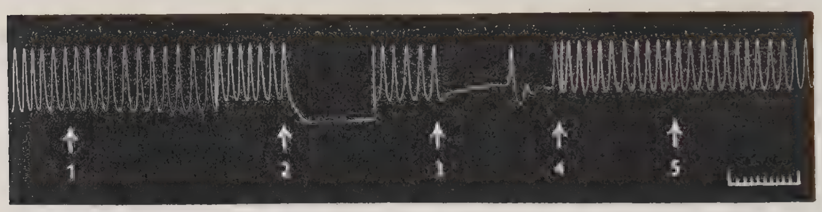

Fig. 1. Records of the action of acetylcholine ACh on the isolated heart of *Cyprina islandica*. (1) ACh 10^{-9} g/ml.; (2) ACh 10^{-8} g/ml.; (3) ACh 5×10^{-9} g/ml.; (4) Mytolon 10^{-6} g/ml. for 10 min, drum stopped; (5) ACh 10 ^{8} g/ml. Kymograph stopped and heart washed between each test. Time units = 6 sec and 1 min.

Mytolon, a neuromuscular blocking agent in vertebrates, is the most effective acetylcholine antagonist known for the *Venus* heart (Luduena & Brown, 1952; Welsh & Taub, 1953). It is also highly effective as an antagonist to acetylcholine on the *Cyprina* heart. In Fig. 1 the action of 10^{-8} acetylcholine is recorded before mytolon was added to the bath and again after the heart had been exposed to 10^{-6} mytolon for 5 min. This concentration of mytolon completely antagonized a dose of acetylcholine that earlier had caused diastolic stoppage. Treatment of a heart with mytolon makes it possible to estimate the amount of excitor substance in an extract when acetylcholine is also present.

The *Venus* heart responds to 5-hydroxytryptamine with an increase in both frequency and amplitude (Welsh, 1953*b*). The same is true for the *Cyprina* heart (Fig. 2). Threshold is near 10^{-10} 5-hydroxytryptamine, but in contrast to acetylcholine action there is a graded response over a wide concentration range. Certain of the ergot alkaloids have a remarkable excitatory action on the *Venus* heart (Welsh & Taub, 1948; Welsh, 1953*b*). LSD is a synthetic derivative of the ergot alkaloids. Since LSD had been shown by Gaddum (1953) to be an effective antagonist for 5-hydroxytryptamine on certain vertebrate smooth muscles it was tested on the *Cyprina* heart. It proved to be a very satisfactory antagonist, as may be seen in Fig. 2.

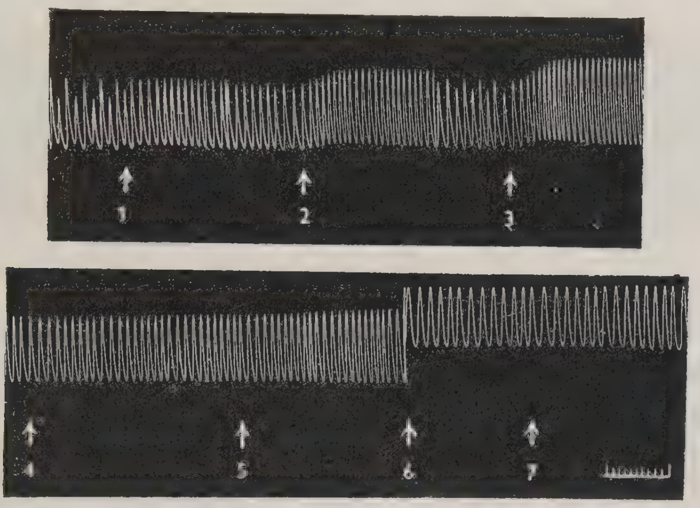

Fig. 2. Records of the action of 5-hydroxytryptamine (5-HT) on the isolated heart of *Cyprina*. (1) 5-HT 5×10^{-9} g/ml.; (2) 5-HT 5×10^{-8} g/ml.; (3) 5-HT 5×10^{-7} g/ml.; (4) LSD 10^{-6} g/ml.; (5) 5-HT 5×10^{-8} g/ml.; (6) LSD 10^{-6} g/ml.; (7) 5-HT 5×10^{-7} g/ml. Heart washed after each test with 5-HT. Time units = 6 sec and 1 min.

From these results, obtained with a limited number of hearts, it is suggested that the *Cyprina* heart (ventricle) should be a useful object for the quantitative estimation of small amounts of acetylcholine and 5-hydroxytryptamine in tissue extracts and biological fluids. Also, it appears probable that the *Cyprina* heart, like the *Venus* heart, is doubly innervated, with the inhibitor nerves releasing an acetylcholine-like substance and the excitor nerves a 5-hydroxytryptamine-like substance.

The Buccinum *heart*

Acetylcholine produces a decrease in amplitude of beat of the *Buccinum* heart with little effect on frequency (Fig. 3). The threshold is below 10^{-9}, and complete inhibition occurs at about 10^{-8} acetylcholine. Mytolon is an effective acetylcholine antagonist (no record of its action is illustrated).

The threshold to 5-hydroxytryptamine is between 10^{-10} and 10^{-9}. A graded response occurs over a wide range of concentrations. The response may be an increase in amplitude with little change in frequency or rise in base-line (Fig. 4), or there may be a marked rise in base-line and increase in frequency as seen in Fig. 5. This variation in response is largely due to the manner of perfusion. A heart from which the perfusion fluid escapes readily is less likely to shorten and beat in a fast, irregular manner, even when strongly excited by 5-hydroxytryptamine.

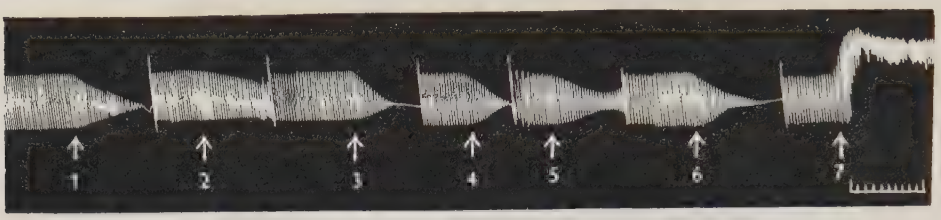

Fig. 3. Records of the actions of extracts of *Buccinum* ganglia and salivary glands compared with acetylcholine (ACh) on the isolated heart (ventricle) of *Buccinum*. (1) Extract of *Buccinum* ganglia made with eserinized sea water. Active material from one milligram of tissue per millilitre of perfusion fluid; (2) ACh 10^{-9} g/ml.; (3) ACh 10^{-8} g/ml.; (4) ACh 5×10^{-9} g/ml.; (5) ACh 2×10^{-9} g/ml.; (6) *Buccinum* ganglion extract as in record 1; (7) extract of salivary glands of *Buccinum* made with sea water (no eserine). Active material from 1 mg tissue/ml. perfusion fluid. Time units = 6 sec and 1 min. (From these records, if the action of the ganglion extract is taken to be equal to 5×10^{-9} AChCl, *Buccinum* ganglia may be considered to contain the equivalent of 5 μg AChCl/g tissue.)

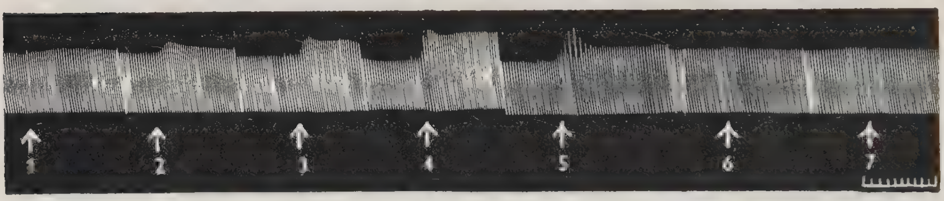

Fig. 4. Records of the action of 5-hydroxytryptamine (5-HT) on the isolated *Buccinum* heart and the blocking action of lysergic acid diethylamide (LSD). (1) 5-HT 5×10^{-9} g/ml.; (2) 5-HT 5×10^{-8} g/ml.; (3) 5-HT 5×10^{-7} g/ml.; (4) 5-HT 5×10^{-6} g/ml.; (5) LSD 10^{-5} g/ml.; (6) 5-HT 5×10^{-7} g/ml.; (7) 5-HT 5×10^{-6} g/ml. Time units = 6 sec and 1 min.

LSD blocks the action of 5-hydroxytryptamine on the *Buccinum* heart. While 5-hydroxytryptamine is readily washed out of the heart, LSD is not. From a concentration of 10^{-5} LSD in the bath, the amount adsorbed by the heart in a period of a few minutes is such that after prolonged washing the excitor action of added 5-hydroxytryptamine is effectively abolished (Fig. 4).

Cardio-excitor and inhibitor substances in molluscan tissues

When the paired, visceral ganglia of *Venus mercenaria* are stimulated electrically, the heart beat is depressed or the heart stops, depending on the voltage and frequency of the stimuli. Prosser (1940) presented evidence that this inhibition of the *Venus* heart resulted from the release of acetylcholine from endings of cardio-inhibitor neurons that had their cell bodies in the visceral ganglia. The results from stimulating isolated hearts led Welsh & Slocombe (1952) to a similar conclusion.

Table I. Acetylcholine Content of Ganglia and Hearts of Molluscs

Class of Mollusc	Species	Tissue	ACh μg/g	Reference
Gastropoda	*Helix pomatia*	Ganglion 'cérébroides'	12	Corteggiani (1938)
	Haliotis tuberculata	Ganglion 'cérébroides'	20	Corteggiani (1938)
	Aplysia depilans	Peri-oesophageal	2–3	Bacq (1935)
	Busycon canaliculatum	Pooled ganglia, except visceral	3–9, av. 5·5	Welsh (unpublished)
Lamellibranchiata	*Venus mercenaria*	Pooled ganglia	1–5, av. 2	Welsh (unpublished)
Cephalopoda	*Octopus vulgaris*	Cerebral ganglia	77	Bacq (1935)
	O. vulgaris	Cerebral ganglia	90	Corteggiani (1938)
	Sepia officinalis	Cerebral ganglia	80	Corteggiani (1938)
Gastropoda	*Aplysia depilans*	Heart	0·2*	Vincent & Jullien (1938)
	Helix pomatia	Heart	2·5–5	Vincent & Jullien (1938)
	Limnaea stagnalis	Heart	5·3–5·5	Vincent & Jullien (1938)
	Murex brandaris	Heart	21	Vincent & Jullien (1938)
	M. trunculus	Heart	23–35	Vincent & Jullien (1938)
Lamellibranchiata	*Anodonta cygnea*	Heart	0·3–0·4	Vincent & Jullien (1938)
	Ostrea edulis	Heart	0·7	Vincent & Jullien (1938)
	Mytilus galloprovincialis	Heart	0·12	Vincent & Jullien (1938)
	Venus mercenaria	Heart	0·1	Welsh (unpublished)
Cephalopoda	*Octopus vulgaris*	Heart	0·1	Vincent & Jullien (1938)
	O. vulgaris	Heart	0·2	Bacq (1935)
	Sepia officinalis	Heart	0·1	Vincent & Jullien (1938)

* See Vincent & Jullien (1938) for values from other species and by a different method of extraction.

Nerve tissue from a variety of molluscs has been shown to contain acetylcholine (or a closely related substance). If the mollusc heart is innervated by cholinergic neurons it, too, should yield acetylcholine. This appears to be true. In Table I will be seen some of the published values for acetylcholine equivalents in molluscan nerve tissue and hearts as well as some unpublished values obtained by the author.

When the cerebral ganglionic mass of *Buccinum* was extracted in eserinized sea water, a heart-depressing substance was found to be present (Record 1, Fig. 5). This substance had an action on the *Buccinum* heart resembling that of acetylcholine. When a portion of this same extract was brought to pH 9–10 with NaOH and boiled for a few minutes, its effect on the heart was excitatory (Record 2, Fig. 5). The excitor action of the extract closely resembled that of 5-hydroxytryptamine.

If there are two sets of cardio-regulator nerves innervating the molluscan heart, demonstration of excitor and inhibitor substances in extracts of heart tissue might be possible. Extracts of hearts (ventricles and auricles) of *Buccinum*, *Pecten* and *Mytilus* were found to have excitor or inhibitor actions on the *Buccinum* heart depending on the method of preparing the extract.

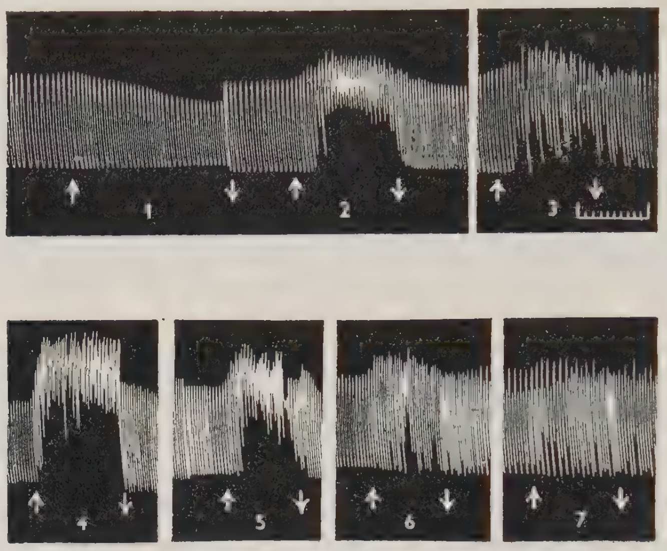

Fig. 5. Records of the action of extracts of *Buccinum* ganglia and salivary glands compared with 5-hydroxytryptamine (5-HT) on the isolated *Buccinum* heart. (1) Extract of ganglia made with eserinized sea water; active material from 1 mg tissue per ml. of perfusion fluid. (2) Part of same extract as in record 1 after bringing to pH 9–10 and boiling. (3) Extract of salivary glands made with sea water (no eserine); active material from 0·1 mg tissue per ml. perfusion fluid. (4) 5-HT 5×10^{-7} g/ml. (5) 5-HT 5×10^{-8} g/ml. (6) 5-HT 5×10^{-9} g/ml. (7) 5-HT 5×10^{-10} g/ml. Time units = 6 sec and 1 min.

5-Hydroxytryptamine occurs in considerable amounts in the posterior salivary glands of *Octopus vulgaris* and *Eledone moschata* (see Erspamer, 1954, for references). When the salivary glands of *Buccinum* were extracted in such a manner that acetylcholine would be destroyed by cholinesterase or by alkaline hydrolysis, the extracts were found to have a marked excitor action on the *Buccinum* heart (Record 3, Fig. 5). The action resembled that of 5-hydroxytryptamine. The excitor activity of a given weight of salivary gland tissue was nearly ten times as great as that of an equal weight of ganglion tissue. Further experiments would be necessary to determine the true nature of the heart-exciting material from the salivary glands of *Buccinum*.

DISCUSSION

It has long been recognized (e.g. Carlson, 1905*a*, *b*) that molluscan hearts are innervated by cardio-regulator nerves. Carlson concluded that the innervation could be single and either excitatory or inhibitory, or, in some species, double with both sets of fibres apparently present. If opposing sets of fibres ran together in the same nerve, as they may in molluscs, it would be difficult to demonstrate double innervation by stimulation experiments. For example, it has long been held that the heart of *Venus mercenaria* is innervated only by inhibitor nerves (Budington, 1904). Actually, following stoppage of the *Venus* heart by electrical stimulation of the visceral ganglia, there is often a much increased frequency and amplitude of heart beat during the recovery period. If an agent is used to block the action of the inhibitor nerve, one then sees only excitation of the *Venus* heart upon stimulation of the visceral ganglia (Welsh, 1953*b*). Such observations have led to the conclusion that the *Venus* heart is doubly innervated and when the mixed nerves to the heart are stimulated the cardio-inhibitory action is normally dominant and tends to mask the simultaneous action of cardio-excitatory neurons. It is quite possible that in some species cardio-excitation is dominant.

When Fredericq in 1947 reviewed the literature on cardio-regulatory nerves in invertebrates, he concluded that the cardio-inhibitor nerves of *Venus* probably released acetylcholine and that the cardio-accelerator nerves of *Aplysia* and of cephalopods probably released adrenaline. He further concluded that the cardio-moderator nerves of cephalopods might possibly act through the release of tyramine. From the present study, incomplete as it is, it appears probable that there are cholinergic inhibitor fibres to the heart of *Buccinum* and perhaps *Cyprina* as well. On the basis of this study it can only be said that possibly the cardio-excitor neurons of *Buccinum* and *Cyprina* act through the release of 5-hydroxytryptamine. The fact that this indole amine has been identified in nervous tissue of *Venus mercenaria* and the whelk, *Busycon canaliculatum* (Welsh, 1954*b*), lends support to this view. Twarog (1954) has also shown that 5-hydroxytryptamine is present in the anterior byssus retractor muscle (nerve endings?) of *Mytilus edulis*, where it perhaps acts in the mediation of inhibitory nerve impulses to this muscle.

The true nature and role of the cardio-excitor substance from the salivary glands of *Buccinum* remain to be determined. Since it acts like 5-hydroxytryptamine and since 5-hydroxytryptamine relaxes certain non-cardiac smooth muscle in molluscs (cf. Twarog, 1954), it is possible that the salivary secretion of *Buccinum* acts, in part, to relax its molluscan prey. The posterior salivary glands of *Octopus vulgaris* contain very large quantities of 5-hydroxytryptamine (Erspamer & Asero, 1952; Bacq, Fischer & Ghiretti, 1952), which is responsible for some of the toxic properties of the saliva of this species.

This paper is based on studies made at the Plymouth Laboratory while the author held a fellowship from the John Simon Guggenheim Memorial Foundation. I wish to express my deep appreciation to the Director and Staff of the Plymouth Laboratory for the excellent facilities placed at my disposal and for their kind hospitality and co-operation.

SUMMARY

Among the larger molluscs available in the Plymouth region, *Cyprina islandica* and *Buccinum undatum* have hearts which, when isolated, are suitable for the bioassay of acetylcholine and 5-hydroxytryptamine.

Mytolon, a vertebrate neuro-muscular blocking agent, is a highly effective antagonist of acetylcholine in the hearts of *Cyprina* and *Buccinum*. Lysergic acid diethylamide (LSD), a synthetic ergot alkaloid derivative, is an effective antagonist of 5-hydroxytryptamine in these hearts.

The nervous system of *Buccinum* contains a cardio-inhibitor material that resembles acetylcholine in its action on the *Buccinum* heart. It also contains a cardio-excitor material whose action on the heart resembles 5-hydroxytryptamine.

Hearts of *Pecten*, *Mytilus* and *Buccinum* also yield (from nerve endings?) similar cardio-excitor and inhibitor principles.

Salivary glands of *Buccinum* contain large amounts of a substance that has an action on the *Buccinum* heart resembling that of 5-hydroxytryptamine.

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THE ACCUMULATION OF RADIOACTIVE IODINE BY *AMPHIOXUS*

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(Text-figs. 1–4)

The endostyle of *Amphioxus* has for a long time been regarded as homologous with the subpharyngeal gland of the cyclostome ammocoete larva. This view was promulgated by Müller (1873) and by Dohrn (1886), and has been supported by many workers, for example Leach (1944). The metamorphosis of portions of the subpharyngeal gland to the thyroid gland of the adult lamprey was followed by Marine (1913), and uptake of radioactive iodine by certain cell elements of the subpharyngeal gland was demonstrated by Gorbman & Creaser (1942). At the same time the latter workers failed to establish the presence of iodine in the endostyle of *Amphioxus* by autoradiographic techniques. Gorbman (1941), however, showed that the ascidian *Perophora annectans* could accumulate radioactive iodine in its pharyngeal stolon but not in its endostyle. The part of the pharyngeal stolon involved is derived from the endostylar region of the pharynx. Recently, Sembrat (1953) has shown that endostyles of *Amphioxus* implanted into larval axolotls accelerated their metamorphosis, and concludes from this that the organs contain an 'active substance (or substances) which may evoke amphibian metamorphosis similarly to the active hormone of the thyroid gland. It is probable that the substance is not identical with thyroxin which may be inferred from the fact that the endostyle of *Branchiostoma* does not accumulate radioactive iodine (Gorbman & Creaser, 1942) as well as from its different and tolerably little efficacious influence on the amphibian metamorphosis as compared with typical metamorphic symptoms induced by the thyroid gland.' The work about to be described has demonstrated the presence of iodine in the endostyle, and suggests, further, that it is present in the form of a 'thyroid hormone-like' substance, thereby supporting Sembrat's general conclusion.

This work was done at the Laboratory of the Marine Biological Association at Plymouth. The author's thanks are due to the Director for making table space and equipment available and to the staff for many kindnesses. His thanks are especially due to Dr D. B. Carlisle for healthy criticism and comment on the work throughout its progress. Miss E. A. Robson of the Zoology Department of the University of Cambridge has assisted greatly with the photographing of the autoradiographs.

MATERIAL AND METHODS

Amphioxus (*Branchiostoma*) *lanceolatus* (Pallas) used in this work were dredged from the Eddystone Grounds off Plymouth. They were kept in circulating sea water in their native shell gravel at the Laboratory of the Marine Biological Association at Plymouth, and maintained thus for at least 1 week, usually several weeks, before being subjected to treatment with radioactive iodine. In all the experiments to be described, carrier-free ^{131}I (as sodium iodide) was used at a concentration of 2 μc/ml. for 15–17 h, at temperatures between 5 and 10° C. The animals were fixed in Bouin-in-seawater, washed thoroughly to remove excess radioactive material, dehydrated, embedded in paraffin wax and sectioned at 10 μ. Sections were mounted on gelatinized slides according to the technique of Doniach & Pelc (1950), dried, washed in xylol to remove the wax, hydrated and covered with Kodak Autoradiographic Stripping Emulsion. The normal exposure time was 14 days. Slides were developed in Kodak D 19*b* developer, fixed and washed in the usual way. Some slides were stained, either with haemalum or Ehrlich's haematoxylin through the gelatin layer of the emulsion. D.P.X. was used as a mounting medium.

EXPERIMENTAL RESULTS

No uptake of ^{131}I has been noted in the prepharyngeal part of the body, in the velum or in the peripharyngeal bands. In the endostyle two longitudinal bands of activity appear, extending from within a millimetre of its anterior extremity to its posterior extremity. In transverse sections they are seen to be situated near the periphery of and slightly lateral to the lateral series of mucous glands (Fig. 1). The two median series of mucous glands show no activity at all. In some sections of the endostyle, these peripheral centres can be seen to consist of two regions, one of which is fairly compact and flattened, outside, but closely applied to its wall, while the other is more diffuse and lies immediately inside the endostylar wall. The two regions are connected by a 'neck' which lies within the cell walls (Fig. 1). The outer region may be extended along the surface of the endostyle laterally on to the lowest gill bar. This activity is undoubtedly associated with mucus secreted by the lateral mucous glands. The inner region, immediately inside the cell walls of the endostyle, is slightly lateral to the lateral mucous glands. In some sections (Fig. 2) several spots of activity can be seen within the lateral mucous glands, near their bases and their borders with the layer of non-mucous cells which separate them from the median mucous glands.

Radio-activity in the secreted mucus can frequently be traced, especially in the posterior part of the pharynx, up the pharyngeal wall and into the epipharyngeal groove. From thence it can be followed backwards into the oesophagus, the mid-gut (Fig. 3) and the hind-gut. The radioactive mucus does not, however, form a continuous cord throughout the length of the gut, but appears in smaller or larger isolated patches. Food particles in the gut, too, having taken up some of the ^{131}I, often form autoradiographic images. No radioactivity either in mucus or in food particles has been found in the lumen of the mid-gut diverticulum.

Radioactive iodine has also been located in the walls of the alimentary canal. The oesophagus may show very slight traces of uptake, but in the mid-gut, mid-gut diverticulum and hind-gut, uptake is much stronger, though never as intense as it is in the endostyle. In the wall of the mid-gut (Fig. 3) and hind-gut it forms a fairly well-defined band near the lumen but separated from the luminar wall by a clear space. This band, in control specimens not treated with ^{131}I, is frequently pigmented yellow or brown and may contain food particles. In the mid-gut diverticulum (Fig. 4) radioactivity in treated specimens is confined to the walls and is found throughout the length of the organ, though less strongly in the anterior part. Within the cells it is distributed mainly in their distal halves. In the ventral wall the activity tends to be concentrated in the middle, and near the distal border of each cell, but in the lateral and dorsal walls it is more evenly distributed, with a slight concentration near the distal border. The reason for the accumulation of iodine in the diverticulum is obscure, unless it is either associated with the mucus of that organ or unless the diverticulum is an additional region for assimilation of the

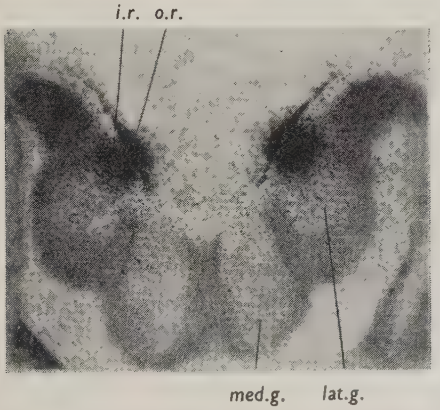

Fig. 1.

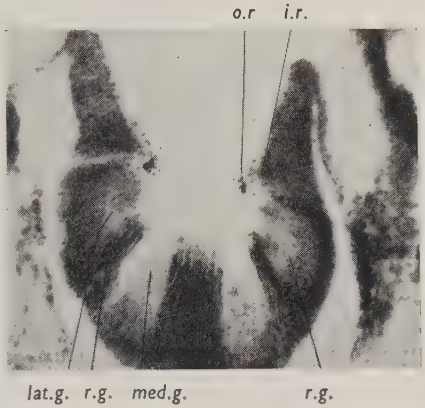

Fig. 2.

Fig. 1. Autoradiograph of a transverse section of the endostyle of *Amphioxus* stained with haemalum; × 300. *lat.g*, lateral mucous gland; *med.g.*, median mucous gland; *o.r.*, outer region of iodine uptake; *i.r.*, inner region of iodine uptake.

Fig. 2. Autoradiograph of transverse section of the endostyle of *Amphioxus* stained with Ehrlich's haematoxylin; × 300. *r.g.*, iodine uptake in body of lateral mucous gland; *lat.g.*, lateral mucous gland; *med.g.*, median mucous gland; *o.r.*, outer region of iodine uptake: *i.r.*, inner region of iodine uptake.

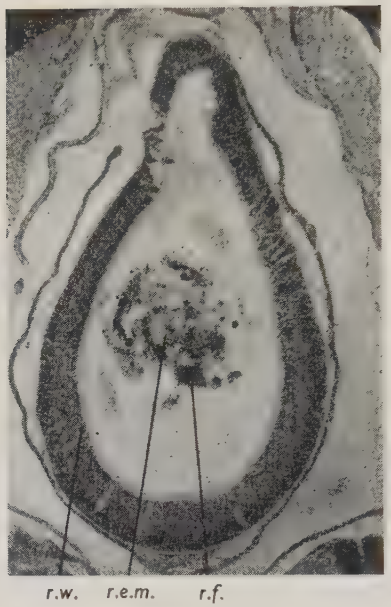

Fig. 3. Autoradiograph of a transverse section of the mid-gut of *Amphioxus* stained with haemalum; ×60. *r.f.*, radioactive iodine associated with food particle; *r.e.m.*, radioactive iodine associated with endostylar mucus; *r.w.*, radioactive iodine in wall of mid-gut.

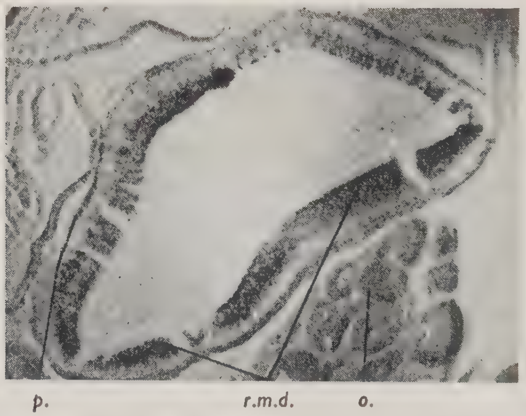

Fig. 4. Autoradiograph of a transverse section of the mid-gut diverticulum of *Amphioxus* unstained; ×84. *r.m.d.*, radioactive iodine in wall of mid-gut diverticulum; *o.*, ovary; *p.*, pigment granules in basal membrane of mid-gut diverticulum.

element. If the latter supposition is true, the iodine must be assimilated directly from the sea water as, according to Barrington (1937), very few food particles find their way into the diverticulum, and those which do are usually quickly swept out again by strong ciliary currents.

A further region of the body where iodine uptake has been observed is in the ventral epithelium of the body wall between the metapleural folds and occasionally extending on to the folds themselves. Here radioactivity is weak and diffuse though it is confined within the limits of the epidermal cells. Van Weel (1937) has observed that cells in this region may have an excretory function. Carbon and melanin particles with which he fed the animals were taken up by amoebocytes in the region of the gut, and then carried by these cells through the blood stream or the coelome to the ventral body wall and deposited in the epithelium or in its underlying connective tissue. From here they were presumably released from the body. The radioactivity in this region is limited to the epithelium. None has been observed in the connective tissue in the vicinity.

Effect of Goitrogens on the Distribution of Radioactive Iodine

The most obvious centre of radioactivity in ^{131}I-treated animals is in the mucus of the lateral mucous glands of the endostyle. The possibility of its being merely adsorbed as inorganic iodine on to the mucus was considered, and to check this point specimens were treated with thiourea or thiouracil simultaneously with their treatment with ^{131}I. Thiourea was used in a concentration of 0·01 % and thiouracil in a concentration of 0·04 %. In the higher vertebrates these and some other similar substances are known to inhibit the formation of the thyroid hormone.

In the concentrations used, thiourea and thiouracil were found to be in no way detrimental to the animals in the period of 15–17 h during which they were subjected to it. Both goitrogens had the same effect. In animals so treated, no radioactivity appeared in the endostyle, in the mucus in the lumen of the gut or in the ventral epithelium. It was still apparent, however, in food particles in the gut and in the walls of the mid-gut, the mid-gut diverticulum and the hind-gut. Its distribution in these organs was the same as in control specimens without goitrogens.

Discussion and Conclusions

Experiments with goitrogens indicate that the sites for assimilation of iodine are in the walls of the mid-gut, the hind-gut and possibly the mid-gut diverticulum. Here it might be taken in either directly from the water in the gut or in association with food particles as intracellular digestion has been postulated by Barrington (1937) and others. Barrington also assumes, as the result of feeding experiments with minute particles of gold, carmine and indian ink, the transference of food particles by the blood stream from the gut to the diverticulum. Iodine might be transferred in the same way. The volume of sea water which passes into the gut is probably small, and as the mucus in the gut of animals treated with goitrogens appears to be free of iodine it follows that most of the iodine entering the gut wall does so in association with food particles. In higher vertebrates iodine taken in through the gut walls is transferred by the blood stream to the thyroid gland, so it is reasonable to assume a similar transference from the gut to the endostyle in *Amphioxus*. This view is supported by the experiments with goitrogens which inhibit its appearance in the endostyle but which do not interfere with its occurrence in the gut wall.

In the endostyle 'fixation' of the iodine occurs in the lateral mucous glands near their inner borders and their bases (Fig. 2), to form a 'thyroid hormone-like substance'. This is associated with the mucus produced by these glands and its formation is prevented by goitrogens. This substance is then transferred to the periphery of the gland and secreted with the mucus. At this point, then, the iodine would be most concentrated, as is apparent from the autoradiographic images. The mucus, once secreted, would become diluted by imbibition of water. No function can at present be ascribed to this hypothetical 'thyroid hormone-like substance', though it may be significant that *Amphioxus* undergoes a marked metamorphosis involving alteration from a very assymmetrical larva to an externally symmetrical adult. All the specimens of *Amphioxus* studied have been fully adult and sexually mature. No work has yet been done on larval forms.

The absence of iodine in the ventral epithelium of specimens treated with goitrogens suggests that the iodine normally located in those cells is derived from iodine 'fixed' in the endostyle. Van Weel (1937) has described the accumulation of excretory material in the ventral epithelium, and assumes that waste food matter can be disposed of by this means. Unless the radioactive iodine remained attached to food particles throughout the digestive processes it is difficult to account for the iodine associated with food appearing in the ventral epithelium. The concentration of ^{131}I used in these experiments (2 μc/ml.) is low, being only about one-twentieth the concentration of the stable isotope normally present in sea water. The animals were thus not subjected to abnormally high concentrations of iodine (^{131}I plus ^{127}I) during the experiments such as might bring about the excretion of abnormally large quantities through the ventral epithelium.

In this connexion an observation made during the course of the experimental work is worthy of note. It was found that animals subjected to treatment with radioactive iodine within 24 h after being caught took up no detectable quantity of iodine. When kept in the circulating water of the laboratory, the amount taken up increased to reach a maximum in about 7 to 10 days. Unfortunately no figures are available for the iodine content of Plymouth Laboratory circulating water, nor for Plymouth coastal waters; but the former value is likely to be low, and a continued sojourn in the Laboratory water might deplete *Amphioxus* of its iodine and make it more ready to take up ^{131}I when that becomes available. *Amphioxus* has been kept alive in circulating water for more than 2 months, however, without apparent detriment. This observation might account for the failure of Gorbman & Creaser (1942) to locate radioactive iodine in *Amphioxus* if they worked with freshly caught material or if their animals were kept in the laboratory in normal sea water before being subjected to iodine treatment.

Two points of interest arise from the results noted in this work. First, the association of iodine with the endostylar mucus, which, of course, passes directly into the gut, may link up with the fact that the thyroid hormone in the higher vertebrates is the only hormone which retains a high degree of effectiveness when administered orally. The secretions of the gonad and the adrenal cortex have a much reduced effectiveness when given by mouth, while the secretions of the remaining endocrine glands are quite ineffective when administered by this path.

The second point concerns the 'colloid substance' which is contained in the alveoli of the thyroid gland of vertebrates. This homogeneous, viscid fluid is the stored secretion of the gland and thus contains the bulk of the iodine present. This colloid substance is, in the author's opinion, the direct evolutionary successor to the endostylar mucus of a protochordate ancestor.

SUMMARY

The localization of iodine has been studied in *Amphioxus* using radioactive iodine and autoradiographic techniques. The most intense concentration was found near the lateral mucous glands of the endostyle where an iodine-containing mucus is secreted into the pharynx. Iodine was also found in the bodies of the lateral mucous glands, in the walls of the alimentary canal and mid-gut diverticulum and in some cells of the ventral body wall. It was found associated with food particles and with mucus in the lumen of the alimentary canal but not in the lumen of the mid-gut diverticulum.

Thiourea and thiouracil inhibit the appearance of iodine in the endostyle, in mucus in the gut, and in the ventral body wall, but do not prevent its appearance in the walls of the alimentary canal and mid-gut diverticulum. The significance of these results is discussed.

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FLUCTUATIONS IN THE DISTRIBUTION AND ABUNDANCE OF INTERTIDAL BARNACLES

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(Text-fig. 1)

It is well known that towards the limits of an animal's geographical range both its abundance and exact boundaries may fluctuate from time to time. These fluctuations can sometimes be correlated with environmental changes, and thus may help to assess the relative importance of the factors that control distribution. For example the distribution of the common intertidal barnacles *Chthamalus stellatus* (Poli) and *Balanus balanoides* (Linnaeus) underwent changes which were attributed to a general rise in temperature over several years (Southward & Crisp, 1954*a*). The abundance of these barnacles has fluctuated further since 1951–52, and we are now able to analyse more closely the relation between the population changes and environmental variations.

Geographical Distribution

The present distribution of *Chthamalus stellatus* and *Balanus balanoides* illustrates very well Darwin's (1872) contention that where two allied species of different range have similar habits and habitats, the territory common to both is small compared with the areas occupied exclusively by either species. Thus, while on the eastern side of the Atlantic *Chthamalus* extends to the tropics and *B. balanoides* penetrates well inside the Arctic circle, the two occur side by side only in the British Isles and northern France. In the course of a general survey aimed at establishing on a quantitative basis the present distribution of some common intertidal animals, we have during the past few years determined carefully the distribution and boundaries of these barnacles in the area common to both. We give here (Fig. 1) the present boundaries of these species in Europe, with our observations grouped into three classes, based on the numerical abundance of the animals: more detailed information has been, and will be, given elsewhere (Southward & Crisp, 1954*b*).

The advantage of demonstrating the limits of a species in terms of objective criteria of abundance rather than by mere records must be stressed. Scattered records of occurrence beyond the margin of measurable decline in population supply little useful information, even when reliable. In the past, too great a reliance has often been placed upon such records, and too often unreliable information (particularly misidentifications and mislabelled museum specimens) has been quoted by several authors in succession without attempt at verification. The number of authors referring to a record is no guide to its value.

Lack of quantitative information has been particularly confusing in the case of *Chthamalus* and *B. balanoides*, which are often mistaken for each other (cf. Darwin, 1854). For example, there appears to be little factual basis for the reported occurrence of *Chthamalus* at Heligoland. The earliest records, as far as can be ascertained from the description (Frey & Leuckart, 1847), refer to *B. balanoides*, while Weltner's (1897) record is based on museum specimens on a mussel-shell, a most unlikely habitat for the barnacle at its limits. The supposed occurrence of *B. balanoides* in the Azores appears to be another example of confusion of these species; Barrois' (1888) account of the Crustacea and Nobre's fauna list (1924) are of dubious value since *Chthamalus* is not recorded at all, while Weltner (1900) gives neither locality nor authority in stating that *B. balanoides* is found in the Azores and Portugal. We have recently examined material collected in the Azores by Dr Chapman, to whom we are indebted for this kindness, and find all to be *Chthamalus stellatus*.

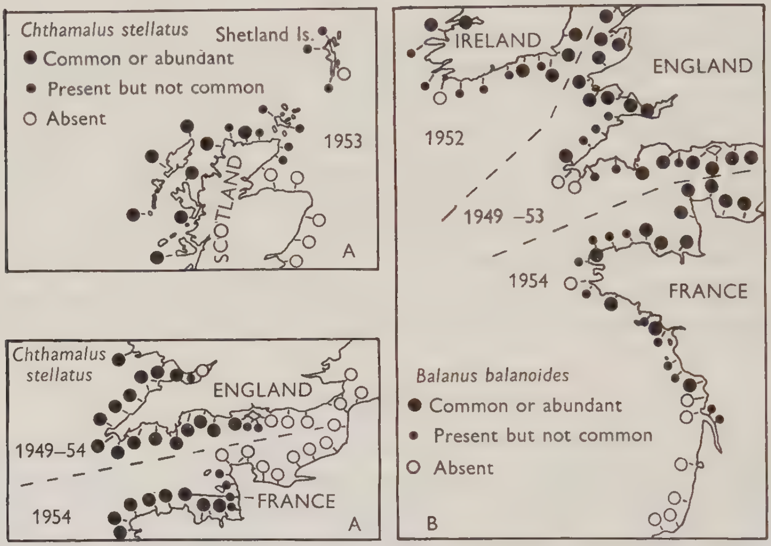
Chthamalus stellatus
Shetland Is.
● Common or abundant
• Present but not common
○ Absent
1953
SCOTLAND
A
Chthamalus stellatus
ENGLAND
1949–54
1954
FRANCE
A
IRELAND
ENGLAND
1952
1949 –53
1954
FRANCE
Balanus balanoides
● Common or abundant
• Present but not common
○ Absent
B

Fig. 1 A. The northern and eastern boundaries of *C. stellatus* in Europe. B: the known southern and western limits of *Balanus balanoides* in Europe. In each species, the dividing line between the present and common categories corresponds to a density of approximately 0·5 individuals per square centimetre.

Even when comparing the present distribution of these barnacles with previous genuine records there is the disadvantage, already mentioned, that with notable exceptions the latter are non-quantitative. However, both Fischer-Piette (1936) and Moore & Kitching (1939) give quantitative evidence from which, by comparison with our observations, the following changes in boundaries may be inferred:

(*a*) Between the 'thirties and 1951 *Chthamalus* advanced eastwards in the English Channel from St Alban's Head and Swanage to the Isle of Wight. The most easterly record is now at Ventnor, while at St Catherine's Point it occurs at a density of 20–30/m^2 at high-water mark. There does not seem to have been any corresponding extension along the coast of France.

(*b*) Between 1936 and 1953 *Chthamalus* spread southwards along the east coast of Scotland from Nybster to Wick, Lybster and Latheron.

(*c*) Between the 'thirties and 1949–51 *B. balanoides* retreated from the south coast of Cornwall. The previous evidence is insufficient to show any changes in the position of the limits elsewhere.

Relative Abundance of the Species

By the end of 1952, as reported in Southward & Crisp (1954*a*), considerable evidence was available that in many parts of Britain there had been a change in the relative abundance of *Chthamalus* and *B. balanoides* since the 'thirties. It now appears that the increases of *Chthamalus* and the decreases of *B. balanoides* reached their extreme in 1951–52, and are at present undergoing reversal. The changes have been followed most closely in Devon and Cornwall, where they have been most obvious, but corroborative evidence is available from other places.

Brixham, South Devon

The densities of the two barnacles at Shoalstone Beach, Brixham, in 1949 and 1952, and each year since 1952 are given in Table I (Appendix). The changes are most clearly brought out by comparing the percentage of *B. balanoides* out of the total population of *Chthamalus* and *B. balanoides*:

	1949	1952	1953	1954	1955
H.W.N.	38	4	5	16	10
M.T.L.	43	14	13	33	25
L.W.N.	93	14	32	43	50

The changes are most marked at L.W.N., where the rate of growth and the mortality are such that both species reach maturity in 1 year, and rarely survive more than 2 years. The evidence demonstrates that although the decline in *B. balanoides* was arrested, and then reversed, from 1953 onwards, the relative proportions of the two species are still different from those existing in 1949. The failure of *B. balanoides* to recover its previous abundance, in spite of some good settlements since 1952, may be accounted for by the continued success of *Chthamalus* in maintaining a population between M.T. level and low water, the intertidal range most favoured by *B. balanoides*.

The Plymouth Area

The results of the observations around Plymouth, at those stations originally chosen by Moore (1936), and at some other places, will be found in Table II (Appendix). In this area the decline in *B. balanoides* up to 1951 was more marked, compared with the 1934 survey, than that which took place at Brixham in 1949–52. The resurgence of the species in the Plymouth area has at some stations been correspondingly obvious, as the following figures (percentages *B. balanoides* out of total *Chthamalus* plus *B. balanoides*) for the estuary of the River Yealm show:

	1934*	1951	1953	1954
H.W.N.	16	<1	<1	1
M.T.L.	87	<1	31	40
L.W.N.	100	<1	9	33

* From Moore (1936).

However, the proportion of *B. balanoides* in the barnacle population of the Plymouth area has not yet reached the level that existed in 1934: at Brixham, where there are no data for the 'thirties, the present proportions of *B. balanoides* are now approaching those of the original survey in 1949.

At some stations in the Plymouth area, e.g. Tinside and Amory Bight, *B. balanoides* is still quite rare. In the Tamar Estuary *Elminius modestus* has remained the dominant barnacle, but at a few other sheltered sites where, for some reason, *Elminius* has always been less common, *B. balanoides* is now equal in number to, or outnumbers, *Chthamalus* at certain levels—e.g. the Breakwater jetty, and Baring Point in the River Yealm.

A most interesting point about the return of *B. balanoides* to Plymouth is that the species reappeared in some wave-beaten sites before other sheltered places. At Church Reef, Wembury, *B. balanoides* settled quite heavily, and survived, on the outermost reefs, as these figures (percentages *B. balanoides* out of total *Chthamalus* plus *B. balanoides*) show:

	1951	1954
H.W.N.	<1	4
M.T.L.	<1	29
L.W.N.	<1	45

while at the shoreward end of the reef it was still as scarce as in 1950–51. Similarly, in 1954, there was a greater density of *B. balanoides* at Amory Bight and on the south side of the Breakwater, than at Tinside on south-facing surfaces which are more sheltered from wave-action. Perhaps more rapid growth and higher mortality at wave-beaten sites causes a greater turnover of established individuals, and hence allows a change in the proportions of the two species to appear more quickly. Alternatively, during a period of increasing abundance larvae may come from elsewhere along the coast, and may therefore settle on the outer reefs first. It is possible that the Yealm Estuary provided a source of such larvae since it retained significant populations throughout the decline (cf. Table II, Baring Point) and was less heavily populated by *Elminius* than other local estuaries.

North Devon and Cornwall

On the northern coast of the Devon and Cornwall peninsula the present distribution of *B. balanoides* is irregular, and our observations are therefore not easy to interpret. An island of fairly high density exists at, and west of, St Ives nearly to Cape Cornwall. East of this *B. balanoides* is mainly restricted to parts of the coast in the neighbourhood of estuaries, for example at Padstow, Boscastle and Bude. Elsewhere it is often uncommon or rare.

Changes in the density of *B. balanoides* have been noticed only in those areas where the species is relatively sparse, and, in common with the changes observed at Plymouth and Brixham, they show a decrease up to about 1951 and an increase since then. For example in 1950–51 at Westward Ho!, Croyde Bay and Trevone, as reported in Southward & Crisp (1954*a*), *B. balanoides* was practically absent, though it was known to have been present at these places in earlier years. Between 1952 and 1954 some settlements took place and at all three stations the species reappeared at low water in small numbers. As elsewhere, however, it is still less common than reported in the 'thirties by Moore & Kitching (1939). At Millook Haven, for example, Moore reported the proportion of 12% *B. balanoides* to 88% *Chthamalus*, whereas in 1955 only 6% *B. balanoides* was found.

In the St Ives–Zennor region investigations were made in 1950 and 1955, and the population was found to be substantially the same. The density of *B. balanoides* was approximately half that recorded by Fischer-Piette (1936).

South Cornwall

B. balanoides was extremely rare to the west of Plymouth from 1950 to 1955. Only sporadic records of isolated individuals have therefore been obtained, and the evidence shows no trend.

Scotland

Since we reported the general increase in *Chthamalus* in the British Isles as a whole we have obtained more definite information on the changes in the Scottish area since the 1930's. Our survey of the Scottish coast was undertaken in 1953, after the reversal of the previous trend, hence the maximum extent of the increase in *Chthamalus* is not known. Table III (Appendix) shows, however, that some increase in *Chthamalus* had occurred between 1934 and 1953, taking Moore & Kitching's observations for the former period as a basis, and expressing ours as nearly as possible in the same form. For many stations, particularly those in Argyll and the Islands, no comparison is possible as the previous observations were insufficiently quantitative. For example, Kitching (1935) reports *Chthamalus* as 'plentiful' at two stations in Mull, one exposed and one sheltered, where we found it to constitute respectively 60 and 7% of the total barnacle population. At Millport, Cumbrae, the density of *Chthamalus* is given by Moore & Kitching as 480/m^2, which is not reconcilable with the figure of 7·5% by weight reported by the same authors for the whole intertidal zone.

In general, the main increase in numbers of *Chthamalus* in Scotland appears to have taken place on west- and south-facing shores.

Irish Sea

Observations on the coasts of the Irish Sea agree generally with those in south-west England. In the Isle of Man, the previous decrease in *B. balanoides* was reversed in 1952, and continued so during 1953–55, although no corresponding decline in *Chthamalus* was noted. At St John's Point, Co. Down, good settlements of *B. balanoides* were noticed on the outer, wave-beaten, reefs in 1953, although in greater shelter *Chthamalus* was still as dominant as it was in 1950 (percentages *B. balanoides* out of total *Chthamalus* plus *B. balanoides*):

	1950	1953
H.W.N.	<1	59
M.T.L.	38	95
L.W.N.	—	83

Along the Lancashire coast, at Blackpool and Rossall, *Chthamalus* has become less common than it was in 1950, but *B. balanoides* is not noticeably more abundant. *Elminius*, on the other hand, has increased considerably and is abundant even at high-water mark. This increase may well have contributed directly to the reduction in *Chthamalus*. At Blackpool in 1953 we found old individuals of the latter being overgrown by younger specimens of *Elminius*. Changes in the foreshore in this area as a result of storm damage and scour may also have had an adverse effect on a species which had only a precarious foothold in the locality.

RELATION TO ENVIRONMENTAL CHANGES

In our previous paper (Southward & Crisp, 1954*a*) we suggested that the changes in the barnacle populations up to 1951–52 were related to the higher sea and air temperatures then prevailing, the southern barnacle being favoured at the expense of the northern species. The recent increase of *B. balanoides* following a period of somewhat lower temperatures (see Tables IV–IX, Appendix) appears to support this view. However, the suggestion of Moore & Kitching (1939) that the distribution of one of these barnacles, *C. stellatus*, was connected with the beneficial effect of water masses of Atlantic origin, has recently been revived by Powell (1954), with reference to its distribution in the north of Scotland. The most marked changes in the barnacle populations have occurred in the south-west of England, where *Chthamalus* is still much more abundant than it was in the 'thirties: yet in this area the proportion of western water, or water of mixed Atlantic and coastal origin, such as had been supposed to favour *Chthamalus*, appears to have continued at the reduced level of the 'thirties (e.g. see Corbin, 1950). The 'Atlantic Water' theory therefore becomes untenable when applied to account for the changes in distribution and abundance.

We shall now consider in more detail how temperature may influence the relative abundance of these two species. In *Chthamalus*, both at Brixham and the Isle of Man, fertilized egg masses, which released nauplii when ripe, were found in the mantle cavity during the breeding period—roughly from May to October—in all the years when observations were made. Therefore temperatures were always sufficiently high to allow the production of young. Nevertheless, summer temperature levels may have some influence on the reproduction. Like *Elminius* and other warm water forms, *Chthamalus* can produce more than one brood of nauplii (Crisp, 1950). It is possible that, as has been demonstrated in *Elminius* (Crisp & Davies, 1955), the interval between broods is shorter at higher temperatures. Thus during a warm summer the number of broods and the larval output would increase directly. Further advantages to the planktonic larvae, the cyprid and the spat would probably result from a warm season, when development and growth would be more rapid, and temperatures closer to those normally experienced by the species at the centre of its geographical range.

Owing to the relatively long period during which *Chthamalus* produces successive broods in the south-west, the developmental stages in the plankton overlap with each other and with the growth of young spat. It is not therefore possible to separate these phases in the life history and to correlate them with temperature anomalies month by month. In 1949 and 1950 (Table IV), however, there was better correlation between annual spatfall and monthly temperature for the months of July–October, than with the months of May and June. This suggests that the temperature requirements of embryonic development, which alone takes place in May and early June, are less exacting than the requirements of planktonic development and the growth of spat.

For the Isle of Man, the evidence is a little more decisive, for the settlement is confined to high water and is restricted to a later period of the year. (Settlement at high water, as distinct from settlement in lower parts of the intertidal zone, occurs later in the south-west also.) There is a good correlation between positive temperature anomalies for any or all months from September to December and good settlements of *Chthamalus* (Table V), rather than for the months from May to August. Taken together, the evidence from Brixham and the Isle of Man points to the need for warmth during the later larval stages and at the time of settlement, and possibly thereafter, rather than during gonad building and embryonic development. However, information over longer periods is desirable.

In the light of the above analysis we may examine mean sea and air temperatures for the months of July to December only, in order to attempt an explanation of the changes in the proportions of *Chthamalus*. At Torquay, from 1946 to the end of 1949, conditions were generally warm, with anomalies above the period mean (Table VI). From 1950 to 1954, there were 2 years, 1950 and 1952, when the temperatures during these 5 months were consistently below the mean, but in the remaining years, the monthly means of sea or air temperature from July till December have been nearly always above the period means. During the same period at Plymouth (Table VII), except for 1950 and 1952, the temperatures were again consistently above the long period mean. Therefore, at these localities, and probably elsewhere in Britain, late summer and autumn temperatures, for 7 out of the 9 years 1946–54, were at a level that should have favoured good settlements of *Chthamalus*. Moreover, the 2 poor years, 1950 and 1952, were separated by a relatively better year. This temperature trend could therefore explain quite well the increase in the species and the continued maintenance of high densities in the south-west, even at low water. On this basis, it could be predicted that there will be no reduction in *Chthamalus* to its former proportions unless there is a succession of colder autumns.

Observations on the settlement of *B. balanoides* at Brixham show no close correlation with winter temperatures as a whole, nor with any particular month (Table VIII). However, over the period 1948–50, when the species was declining, temperatures were generally above the period mean. Since then, with certain exceptions, either sea or air temperatures (or both) have been below the period mean, and settlements, except in 1951, have correspondingly shown a marked increase. There would thus be some correlation of the recent more successful settlements of *B. balanoides* with lower winter temperatures, if some explanation could be given for the anomaly in 1951. It must be remembered that at this time stocks of the species were probably low generally in the south-west, as they were known to be at Plymouth (Table II). Few young individuals were present at this time, for the spatfalls of 1948–50 appear to have failed to survive in any numbers. The old individuals had a very high rate of infection by the castrating parasite *Hemioniscus* (Southward & Crisp, 1954*a*). If the generally colder conditions in the early part of 1951 allowed the survival of the spatfall, small though it was, these younger individuals with, presumably, a lower infection rate of the parasite, would augment the spatfall the following year. A lag of this sort in the recovery of the species would not be surprising in view of its precarious position in the south-west.

At Plymouth the decline of *B. balanoides* during the warm period ending in 1950 reached a much lower level than at Brixham, and it would be correspondingly more difficult for the species to return. Moreover, although since 1951 temperatures below the long period average have occurred in January and February, in March and April, the months when settlement takes place, temperatures have been above the period mean.

The main part of this paper was prepared during the winter and spring of 1954–55. On the basis of the above discussion it was predicted that the persistent cold weather that occurred in the early part of 1955 might favour good settlements of *B. balanoides* in the south-west. The meteorological observations now available show that during the months of January to June 1955, apart from air temperatures for April, temperatures were consistently below the period means (Table X). Sea temperatures were especially low in March and April, when the major (or only) settlement of *B. balanoides* takes place. It is therefore interesting to report that quite a good settlement of *B. balanoides* occurred in south Devon in the spring of 1955. Comparatively heavy spatfalls (1–2/cm^2) were seen at Wembury, the Erme Estuary, and at Prawle Point in April and May. Good settlements were probably general along the whole of the south coast since Stubbings (private communication) reports exceptionally heavy and prolonged spatfalls of *B. balanoides* in the Portsmouth area. In the Menai Straits the settlement was heavier and more prolonged than had been experienced during the past 4 years.

Counts were made in the Plymouth and Brixham area in August 1955, when the spat had reached almost adult size, and these observations showed that increases in the relative proportions of the species had occurred at Brixham and in the Yealm Estuary, though no change was discernible at Plymouth, Tinside (Table X). At Wembury, although the relative proportions of *B. balanoides* and *C. stellatus* on the outer, wave-beaten, reefs were the same as in 1954, settlement had spread to the shoreward reefs from which the species had been completely absent for at least 5 years. Thus at three out of four localities, *B. balanoides* showed an increase in number or range during the cold spring of 1955.

INFLUENCE OF TEMPERATURE

Darwin (1872) cautioned against attributing the distribution of an organism directly to temperature differences in the environment. He pointed out that competition between species would probably be the most exacting factor, but that this would be influenced by conditions of temperature or climate, which favoured one or other of the competitors.

There is little doubt that in south-west Britain, *C. stellatus* and *B. balanoides* are in direct competition over the greater part of the intertidal zone, for the total numbers supported by the environment have remained fairly constant in spite of changes in the proportions of each species. How, then, can temperature influence this competition?

We have already pointed out how warm summer temperatures may increase directly the fecundity and survival of *Chthamalus*, and support of this view is afforded by the heavy spatfalls in certain areas such as Loch Sween, Dundrum Bay, etc. (Southward & Crisp, 1954*b*). Increased fecundity will ultimately lead to increased intertidal cover by the species, and so deny rock space to its competitor. However, no similar beneficial effect of low winter temperature can increase the fecundity of *B. balanoides*, for the eggs are spawned by mid-winter, before the sea and air temperatures reach their minima, and breeding can occur only once a year. Hence, then, low winter temperature can directly favour *B. balanoides* only by delaying embryonic development to a point where the larvae may benefit more from the spring phytoplankton, or, more dubiously, by some effect on the survival or vigour of the larvae or spat.

On the other hand, competition between the species for food is operating continuously and must be especially severe on young spat surrounded by cirral nets of older individuals. Moreover, barnacles will readily feed on newly hatched nauplii of their own or other species (unpublished observations). The rate of feeding of each species, in addition to its direct influence on its own growth and fecundity, will therefore influence competing species both by removal of food and by ingestion of their larvae.

It has been shown that the frequency of cirral beat in each species has an optimal temperature range; in *B. balanoides* it lies between 0 and 18° C, in *Chthamalus* between 5 and 30° C (Southward, 1955). Outside these ranges the species will not be able to feed efficiently. Results of frequency determinations show that after allowing for much individual variation, *B. balanoides* is the more efficient below about 15° C, *Chthamalus* above (Southward, 1955). Therefore the lower the temperature at which the spat of *B. balanoides* are growing, the less the competition to which they will be exposed. On the other hand, above 17° C *B. balanoides* shows reduced activity, while *Chthamalus* shows a rapidly increasing beat. High summer and autumn temperatures should therefore favour the survival of the spat of *Chthamalus*. On this basis the balance between the two species will depend on the temperature variation throughout the year, and their distribution limits would not necessarily correspond with temperature extremes.

Moore & Kitching (1939) minimized the significance of competition as a limiting factor because the zones of adult *Balanus* and of adult *Chthamalus* were occasionally distinct. Our observations agree with most of theirs in that almost everywhere the zones interpenetrate, if only to a slight extent, with no space between them. The sharpness of separation of the two zones where it occurs could be an indication of extreme interspecific competition within each zone; it would also be enhanced by gregariousness on the part of the cyprid larvae (Knight-Jones, 1953), for *Chthamalus* has no appreciable effect in stimulating the setting of *Balanus* (Knight-Jones, 1955).

The view that competition, modified by temperature, is the main factor limiting each species however suggests possible explanations to problems which have been posed by previous authors. It explains the gradual restriction of *Chthamalus*, both in its northern and estuarine limits, to the upper zones where it suffers less competition from *Balanus*. The restriction of *Chthamalus* at its northern limits to very exposed headlands may not be due entirely to the suitability of exposed areas to *Chthamalus*; it may survive there because it is relatively better equipped to withstand severe exposure than is *Balanus*, and so benefits from lack of competition. The nature of the microhabitat, in which it is found under such conditions (viz. slightly sheltered cracks in the rock), suggests that the exposure is not optimal, but almost as severe as the species can endure. In more sheltered localities in most parts of Britain *Balanus* normally dominates completely, but it may be significant that wherever *Chthamalus* is found abundantly in shelter, other than in a narrow zone at high water, *B. balanoides* is scarce. For example, in harbours west of Plymouth, where *Chthamalus* occurs at all levels, *B. balanoides* is scarcely represented. In Loch Sween, *B. balanoides* is absent in the areas of abnormal abundance of *Chthamalus* (cf. Southward & Crisp, 1954*b*). In a similar way, the presence of *Balanus balanoides* in creeks and estuaries in south-west England, and its absence from the open coast, may be attributable to the adverse effect of estuarine conditions on *Chthamalus stellatus*.

We conclude that the distribution of these two species, with their similar habitat and habits, cannot be adequately explained by the action of the physical environment on either of them alone. We suggest, instead, a dynamic concept with the two competing species in a state of equilibrium. Even quite minor changes in the physical environment, favouring one or the other of the species, will, if sufficiently prolonged, cause the boundaries of distribution to alter.

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SUMMARY

Recent observations on the distribution and abundance of the commoner intertidal barnacles indicate that, following a decline up to 1950–51, the northern *Balanus balanoides* is now increasing in abundance. The influence of environmental variations, particularly temperature, is discussed.

It is suggested that the distribution of the two competing species *B. balanoides* and *Chthamalus stellatus*, and the fluctuations in their abundance, are best explained dynamically. The equilibrium existing between them is

altered by changes in the environment, which give one species an advantage over the other. Temperature, which affects the cirral activity of the two species differently, has probably the most important influence on their relative abundance.

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APPENDIX

TABLE I. THE DISTRIBUTION AND ABUNDANCE OF CERTAIN BARNACLES AT SHOALSTONE BEACH, BRIXHAM, 1949–55

ne levels are only approximate. The density is given in numbers per square centimetre, based on counts several different places at each level. Some of the counts made within 5 months of the larval settlement w the numbers of adults and spat separately.)

	March 1949		February 1952		May 1953		April 1954		January 1955	
evel	*C. stellatus*	*B. balanoides*	*C. stellatus*	*B. balanoides*	*C. stellatus*	*B. balanoides*	*C. stellatus*	*B. balanoides*	*C. stellatus*	*B. balanoides*
V.N.	2·76	1·69	5·49	0·25	9·5	0·55+0·40	4·55+7·75	0·85+0·70	3·02+0·28	0·34
T.L.	2·64	1·98	2·80	0·45	6·8	1·05+0·80	2·20+4·15	1·10+1·95	3·14+0·74	1·06
V.N.	0·15	2·00	2·10	0·35	4·8	2·25+1·60	1·60+2·70	1·20+2·70	0·88+0·32	0·87

TABLE II. THE DISTRIBUTION AND ABUNDANCE OF CERTAIN BARNACLES IN PLYMOUTH AREA, 1951–54

(To be compared also with tables in Moore, 1935. The numbers are given as the average per square metre, based on 2–5 counts of areas of 25–100 cm² at each level; the levels at Tinside were referred to the nearest bench mark of the Ordnance Survey, and those on the Breakwater obtained from Southward & Orton (1954), but at the other stations the levels were estimated by reference to the predicted height of the tide at the time; counts of *Chthamalus* made later in the year than September give adults and young specimens separately.)

(*a*) Amory Bight, between Rame Head and Penlee Point

	September 1951	December 1954		
	C. stellatus	*C. stellatus*		*B. balanoides*
Level in metres, relative to O.D.		Adults	Spat	
3·9	450	—	—	—
3·3	2,200	2,200	0	0
2·7	67,600	—	—	—
2·3	—	12,400+	1,000	0
2·1	75,200	—	—	—
1·5	73,600	41,600+	1,400	100
0·9	78,200	—	—	—
0·6	—	40,000+	8,800	1,600
0·2	44,400	—	—	—
−0·2	—	41,600+	56,000	800
−0·4	23,400	—	—	—
−1·0	15,500	28,800+	32,000	200
−1·6	9,600	12,000+	4,000	0

(Table II—*continued*)

(*b*) Plymouth Breakwater, vertical pile of jetty on north side

	September 1951			October 1954		
Level	*C. stellatus*	*B. balanoides*	*E. modestus*	*C. stellatus*	*B. balanoides*	*E. modestu*
M.T.L.	44,800	1,000	1,100	16,000	16,000	1,200

(*c*) Plymouth Breakwater, south side, Traverse B of Southward & Orton, 1954

	September 1951		October 1954		
Level (m)	*C. stellatus*	*B. balanoides*	*C. stellatus*		*B. balanoides*
			Adults	Spat	
3·0	28	0	570	0	0
2·5	12,200	0	33,600+	8,000	0
2·0	9,500	0	35,200+	12,400	0
1·5	46,400	0	48,000+	17,600	0
1·0	37,600	0	37,600+	38,000	2
0·5	30,600	0	38,400+	24,000	80
O.D.	40,200	200	24,600+	36,000	1,100
−0·5	33,200	0	20,800+	22,800	2,800
−1·0	9,400	2	11,600+	8,400	500

(*d*) Tinside, below the laboratory

	September 1951		October 1953			December 1954		
Level (m)	*C. stellatus*	*B. balanoides*	*C. stellatus*		*B. balanoides*	*C. stellatus*		*B. balanoides*
			Adults	Spat		Adults	Spat	
2·28	500	0	800	0	0	500	0	0
1·88	—	—	—	—	—	4,800+	300	0
1·67	28,840	0	26,000+	6,000	0	—	—	—
1·38	—	—	—	—	—	24,000+	8,000	0
1·06	32,400	0	24,800+	32,000	0	—	—	—
0·88	—	—	—	—	—	34,800+	16,000	0
0·45	30,800	0	27,200+	32,000	0	—	—	—
0·38	—	—	—	—	—	24,000+	27,200	0
−0·16	24,000	0	21,200+	24,000	60	34,800+	21,600	200
−0·62	—	—	—	—	—	24,000+	16,800	0
−0·77	19,600	0	—	—	—	—	—	—
−1·12	—	—	—	—	—	2,800+	12,400	0
−1·38	1,150	0	1,350+	500	0	500+	1,600	0
Shaded surfaces at −1·3	—	400	—	—	—	—	—	2,500

(*e*) Henn Point, Tamar Estury

	September 1951			October 1953			December 1954		
Level (m)	*C. stellatus*	*B. balanoides*	*E. modestus*	*C. stellatus*	*B. balanoides*	*E. modestus*	*C. stellatus*	*B. balanoides*	*E. modestus*
1·1	550	—	—	400	—	225	500	—	300
0·4	400	66	2,300	100	100	800	0	800	9,600
0·2	0	500	25,600	0	?	9,200	0	500	18,000
O.D.	0	75	28,200	0	0	16,000	0	?	5,200
−0·2	0	100	9,600	0	0	9,600	—	—	—
−0·7	0	0	8,800	0	0	18,400	0	700	20,800
−1·9	0	0	18,800	—	—	—	0	800	16,800

(Table II—*continued*)

(*f*) Misery Point—Cellar Beach, Yealm Estuary

Level (m)	September 1951 *C. stellatus*	September 1951 *B. balanoides*	October 1953 *C. stellatus* Adults	October 1953 *C. stellatus* Spat	October 1953 *B. balanoides*	December 1954 *C. stellatus* Adults	December 1954 *C. stellatus* Spat	December 1954 *B. balanoides*
3·0	—	—	1,200	0	0	—	—	—
2·7	4,500	0	—	—	—	1,600	0	0
2·4	—	—	20,000+	2,000	0	—	—	—
2·1	46,800	0	—	—	—	80,000+	2,000	1,200
1·5	41,200	100	48,000+	11,200	0	68,000+	24,000	6,000
1·2	—	—	60,000+	13,600	0	—	—	—
0·9	73,600	0	—	—	—	26,400+	27,200	4,800
0·6	—	—	16,000+	18,000	5,500	—	—	—
0·3	30,000	0	—	—	—	—	—	—
O.D.	—	—	16,000+	32,000	7,00	12,000+	32,000	8,200
−0·3	21,200	0	—	—	—	24,000+	16,000	5,600
−0·6	—	—	29,600+	40,000	1,600	3,200		1,600
−0·9	766	0	4,000		400	—		—
−1·5	0	0	—		—	400		800

(*g*) Baring Point, Yealm Estuary

Level	September 1951 *C. stellatus*	September 1951 *B. balanoides*	October 1953 *C. stellatus*	October 1953 *B. balanoides*	December 1954 *C. stellatus* Adults	December 1954 *C. stellatus* Spat	December 1954 *B. balanoides*
H.W.N.	3,700	200	?	?	8,000+	2,000	400
M.T.L.	2,500	1,000	?	2,600	2,000+	4,000	13,600

Table III. Abundance* of *C. stellatus* in Scotland

Place	In 1934–36 (from Kitching, 1935; Moore & Kitching, 1939)	In 1953
Nybster, Caithness	Rare—2 found in 15 min	No change, but 5/m² at Wick
Dunnet Head, Caithness	Rare—6 seen in 10 min	0·2 % (mean of observations on both sides of headland)
Skullomie, Sutherland	0·6 %	0·8 % (but much commoner at Farr Bay, to the east)
Geodha Chobhair, Sutherland	6·4 %	29 %
Seana Chamas, Ross	0·6 %	3 % (on shores to north and south, between 20 and 40 %)
Elgol, Skye	10 %	Not visited; corresponding area on Sleat, 56 %
Easdale, Seil	Scarce	11·4 %
Loch Melfort, Argyll	Scarce	30 %
Ardnoe Point, Argyll	Rather sparse	700/m²
Southend, Kintyre	Absent	7 %

* As numbers found at H.W.N. in a certain time, or numbers per unit area of rock at H.W., or as percentage of total barnacle population over whole intertidal zone.

TABLE IV. SETTLEMENT OF *C. STELLATUS* AT SHOALSTONE BEACH, BRIXHAM, IN RELATION TO TEMPERATURES AT TORQUAY NEARBY

		1949		1950	
Numbers per sq. cm. at end of main settlement (July–October)	H.W.N.	12		5	
	M.T.L.	14		5	
	L.W.N.	28		11	
		Air	Sea	Air	Sea
Monthly anomalies of mean temperature	July	+1·1	+2·2	−0·3	0
	Aug.	+0·5	+0·8	−0·7	−0·1
	Sept.	+2·0	+2·2	−0·5	−0·5
	Oct.	+1·6	+2·1	−0·6	−0·6

Note. Anomalies of sea temperature are relative to the means for the period 1946–54; anomalies of air temperature are relative to the means for the period 1906–35. In this and subsequent tables the anomalies are based on sea and air temperatures supplied by the following authorities: Borough Meteorologist, Torquay; Director, Marine Biological Station, Port Erin; Port Health Officer, Plymouth. Additional values of temperature, including the means of air temperature for the period 1906–35, were obtained from the following: Air Ministry, 1936; Air Ministry, 1946–55: Southward, 1953.

TABLE V. SETTLEMENT OF *C. STELLATUS* AT KALLOW POINT, PORT ST MARY, ISLE OF MAN, IN RELATION TO LOCAL SEA AND AIR TEMPERATURES

		1949		1950		1951		1952		1953	
Numbers per sq. m at 1 ft. above H.W.N. the following spring		24		4		30		0		23	
		Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea
Monthly anomalies of mean temperature	Sept.	+1·9	+1·1	−0·9	0	+0·3	−0·2	−0·9	−0·9	+0·4	+0·5
	Oct.	+1·3	+1·0	−0·3	−0·4	+0·6	+0·1	−2·0	−1·2	+0·4	+0·6
	Nov.	+0·3	+0·5	−1·5	−0·9	+0·9	+0·7	−2·7	−1·0	+2·4	+0·7
	Dec.	+0·6	+0·2	−3·0	−1·2	+0·6	+1·0	−1·3	−1·3	+2·0	+1·2

Note. The anomalies are relative to the means for the period 1947–53.

TABLE VI. ANOMALIES OF MEAN AIR,* AND MEAN SEA† TEMPERATURES AT TORQUAY

	1946		1947		1948		1949		1950		1951		1952		1953		1954	
	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea
Jan.	−0·4	0·2	−1·7	−0·6	1·1	0·9	0·8	0·6	0·1	0·3	0	−0·3	−1·2	0	−1·6	−1·3	−1·0	0·4
Feb.	1·9	1·8	−5·7	−3·0	0·1	0·9	1·2	1·1	1·4	1·1	−0·4	0·2	−1·0	−0·2	−0·9	−0·4	−1·1	−0·9
Mar.	0·6	−0·2	−0·9	−2·3	2·1	0·7	0	0·3	2·2	1·3	−0·9	−0·5	1·1	0·6	−0·7	−0·3	−0·4	0·4
Apr.	1·0	0·8	−0·4	−0·8	0·5	0·3	1·3	0·7	0	0·4	−0·9	−0·5	0·6	−0·6	−0·4	−0·6	−0·5	0·3
May	−0·9	0·2	0	0·6	0·1	0·8	0·1	0·5	0·1	0·4	−1·7	−1·1	0·5	0·3	0·5	0	−0·6	−0·6
June	−1·3	−0·8	0	−0·3	−0·1	−0·5	−0·1	1·2	1·4	0·9	−0·2	−0·3	0	0·9	0·3	−0·5	−0·7	−0·9
July	−0·4	−0·3	−0·1	−0·4	−0·5	−0·2	1·1	2·2	−0·3	0	0·1	0	0·1	0·7	−0·1	−0·4	−2·0	−1·2
Aug.	−0·1	−1·1	2·3	1·9	−0·9	−0·1	0·5	0·8	−0·7	−0·1	−0·7	−0·4	0·3	0·4	0	0	−1·6	−1·3
Sept.	−0·3	−1·0	0·9	1·4	−0·3	0	2·0	2·2	−0·5	−0·5	0·1	−0·5	−2·5	−0·9	−0·3	0	−0·8	−0·6
Oct.	0·5	−0·4	0·6	0·8	0	−0·1	1·6	2·1	−0·6	−0·6	−0·3	−0·2	−1·3	−1·4	−0·3	0·1	1·3	0
Nov.	1·4	−0·3	1·3	1·1	1·9	0·1	0	0·2	−0·4	−1·1	1·4	0·4	−1·7	−0·9	1·4	0·7	0·3	0·2
Dec.	2·1	−0·7	0·1	−0·2	1·1	1·1	0·5	0·5	−3·3	−2·1	1·0	0·6	−1·3	−1·6	2·4	1·8	1·7	0·2
Year	0·1	−0·2	−0·2	−0·3	0·4	0·3	0·8	1·0	0	0	−0·2	−0·3	−0·5	−0·3	0	−0·1	−0·4	−0·3

* Relative to the means for the period 1906–35.

† Relative to the means for the period 1946–54.

TABLE VII. ANOMALIES OF MEAN AIR TEMPERATURE,* PLYMOUTH HOE, AND MEAN SEA TEMPERATURE,† PLYMOUTH TINSIDE

	1946		1947		1948		1949		1950		1951		1952		1953		1954	
	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea
Jan.	−0·3	0·6	−1·9	−0·6	1·0	0·7	1·3	0·7	−0·1	0·6	0	−0·6	−1·3	−0·2	−1·3	−0·3	−1·0	0·1
Feb.	1·8	1·0	−5·7	−2·7	0	0·5	1·3	1·2	1·6	1·3	−0·3	−0·2	−1·1	0·1	−0·9	−0·2	−1·1	−0·4
Mar.	0	0	−0·7	−1·9	3·1	0·1	0·6	0·9	2·4	1·1	−0·8	−0·2	1·0	0·1	0·2	0·3	0·8	0·3
Apr.	1·9	0·9	0·7	0·1	1·4	0·4	1·9	0·9	0·6	0·6	−0·5	−0·5	1·2	0·3	0·6	0·1	−0·3	0·6
May	0	0·3	0·8	−0·2	1·2	0·9	0	0·7	1·2	0·8	−1·0	−0·7	1·5	1·1	1·2	0·5	0	0·1
June	−1·1	0·3	0·5	−0·2	0	0·2	1·9	0·8	1·9	1·3	0·2	−0·1	0	0·4	0·6	−0·6	−0·5	0
July	−0·2	0·4	0·3	−0·4	0	−0·6	2·2	2·0	−0·1	0·4	0·5	−0·1	0·7	0·4	−0·3	−0·3	−1·9	−1·6
Aug.	1·2	−0·5	3·7	1·5	0	0	1·6	0·9	−0·3	0·3	−1·1	−0·2	0	0·4	0·5	0	−0·7	−1·7
Sept.	0	−0·2	1·4	1·6	0·3	−0·2	3·2	1·9	−0·4	0·1	0·1	−0·4	−2·0	−0·4	0·5	−0·2	−0·7	−0·4
Oct.	0·8	0	0·9	1·3	0·4	0·3	2·4	1·3	−0·2	−0·1	0·2	0·1	−0·4	−0·6	0·3	0·5	1·3	0·3
Nov.	2·0	0·3	1·7	1·5	2·2	0·6	0·5	0·7	−0·3	−0·3	1·7	0·4	−1·3	−0·2	2·0	0·8	0·6	0·2
Dec.	−1·6	−0·2	0·2	1·0	1·1	0·8	0·8	−0·4	−3·0	−0·6	0·9	0·6	−0·9	−0·9	2·6	2·1	1·8	0·9
Year	0·1	0·3	0·1	0	0·9	0·3	1·3	1·1	0·3	0·4	0	−0·1	−0·2	−0·1	0·5	0·2	−0·4	−0·1

* Relative to the means for the period 1906–35.

† Relative to the means for the period 1893–1948.

TABLE VIII. THE SETTLEMENTS OF *B. BALANOIDES* AT SHOALSTONE BEACH, BRIXHAM, COMPARED WITH SOME WINTER TEMPERATURES EXPERIENCED AT TORQUAY NEARBY

	1946		1947		1948		1949		1950		1951		1952		1953		1954	
Maximum numbers per sq.cm of rock at end of settlement (March–April)	12		10		8		7·5		3·6		<1		10		6		12	
	*	†	*	†	*	†	*	†	*	†	*	†	*	†	*	†	*	†
Anomalies of temperature:	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea
Previous December	—	—	2·1	−0·7	0·1	−0·2	1·1	1·1	0·5	0·5	−3·3	−2·1	1·0	0·6	−1·3	−1·6	2·4	1·8
January	−0·4	0·2	−1·7	−0·6	1·1	0·9	0·8	0·6	0·1	0·3	0	−0·3	−1·2	0	−1·6	−1·3	−1·0	0·4
February	1·9	1·8	−5·7	−3·0	0·1	0·9	1·2	1·1	1·4	1·1	−0·4	0·2	−1·0	−0·2	−0·9	−0·4	−1·1	−0·9
March	0·6	−0·2	−0·9	−2·3	2·1	0·7	0	0·3	2·2	1·3	−0·9	−0·5	1·1	0·6	−0·7	−0·3	−0·4	0·4
April	1·0	0·8	−0·4	−0·8	0·5	0·3	1·3	0·7	0	0·4	−0·9	−0·5	0·6	−0·6	−0·4	−0·6	−0·5	0·3

* Relative to the period means 1906–35.

† Relative to the period means 1946–54.

TABLE IX. ANOMALIES OF MEAN ANNUAL AIR TEMPERATURE* AT DOUGLAS, AND MEAN ANNUAL SEA TEMPERATURE† AT PORT ERIN, ISLE OF MAN

	1946	1947	1948	1949	1950	1951	1952	1953
Air	0	−0·2	0·2	0·4	−0·2	−0·3	−0·4	0·6
Sea	0·3	−0·7	0	0·7	0·3	−0·2	−0·2	0·2

* Relative to the grand mean for 1906–35.

† Relative to the grand mean for 1903–51.

TABLE X. THE ABUNDANCE OF *B. BALANOIDES* AT CERTAIN LOCALITIES IN SOUTH DEVON, AUGUST 1955, AS NUMBERS PER SQUARE CENTIMETRE OF ROCK, SHOWING THE RELATION OF THE SPRING SETTLEMENT OF SPAT TO SEA AND AIR TEMPERATURES

	Plymouth, Tinside	Wembury, outer reefs		River Yealm, Misery Point		Brixham, Shoalstone	
		Adults	Spat	Adults	Spat	Adults	Spat
H.W.N.	Nil on usual sampling area, up to 0·2 at L.W. in places	0	0·1	0·28	0·54	0·16	0·18
		(2)		(11)*		(12)	
M.T.L.		0·1	1·22	0·48	1·08	0·15	0·94
		(24)		(22)		(45)*	
L.W.N.		0·2	1·4	0	1·13	0·01	1·30
		(43)		(66)*		(89)*	

	Plymouth Hoe temperatures, anomalies of		Torquay temperatures, anomalies of	
	Mean sea† (° C)	Mean air‡ (° C)	Mean sea§ (° C)	Mean air‡ (° C)
January	−0·3	−0·2	−0·4	−0·2
February	−0·1	−2·4	−0·3	−3·0
March	−1·3	−2·2	−2·6	−2·1
April	−1·4	+1·2	−1·0	+0·8
May	−1·2	−1·3	−0·9	−1·9
June	−0·6	+0·2	−0·2	−0·2

The figures in brackets show the percentage of *B. balanoides* out of the total *B. balanoides* and *Chthamalus stellatus* populations.

* Indicates an increase in this percentage compared with 1954.

† Relative to the grand means for the period 1900–55.

‡ Relative to the grand means for the period 1906–35.

§ Relative to the grand means for the period 1947–54.

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A RECORD OF PLANKTON ON THE ECHO-SOUNDER

By D. H. Cushing and I. D. Richardson
Fisheries Laboratory, Lowestoft

(Text-figs. 1–3)

A number of records from echo-sounders have been loosely attributed to the presence of plankton. Only two records have been adequately identified as having been produced by plankton; first, the traces of fish larvae, or shallow scattering layers (Burd & Lee, 1951); and secondly, the echo layer, at the depth of the temperature discontinuity or thermocline, which is sometimes associated with plankton animals or plants (Cushing, Lee & Richardson, in press). A third type of record, that from the 'deep scattering layer', has been associated with the presence of euphausiids (Hersey & Moore, 1948; Moore, 1950; Boden, 1950): an equally plausible association with the presence of fish has been made by Marshall (1951), Tucker (1951) and Hersey & Backus (1954). A fourth type of echo record, a 'noisy' record, will be described and will show that it is probably attributable to plankton organisms, consisting, in one instance, of euphausiids.

MATERIAL AND METHOD

During the work to be described the amplifier was kept at known settings, engines had no effect on our echo-sounders and we carried out most of our work in flat calm weather. We sought to identify a factor in 'water noise' by studying the plankton content of the water, at the time when records of noise were being made.

There are three groups of observations, the first in Windermere, followed by two in the North Sea.

RESULTS AT WINDERMERE

When we were working on Windermere, the lake was flat and calm. During the daytime, noise appeared suddenly at a high level of amplification, as a black band right across the paper. On one night, however, at a lower level of amplification, noise appeared diffusely from the transmission mark downwards, rather as it does in the sea. But the diffuseness faded with depth, which was not characteristic of noise traces that had been taken at sea. The actual trace is shown in Fig. 1.

Because the diffuse noisy trace that faded with depth was only found at night, it was thought to be associated with the presence of plankton animals that had migrated to the surface at night. The phytoplankton makes no such migration, and although present in the lake in dense numbers, was consequently disregarded.

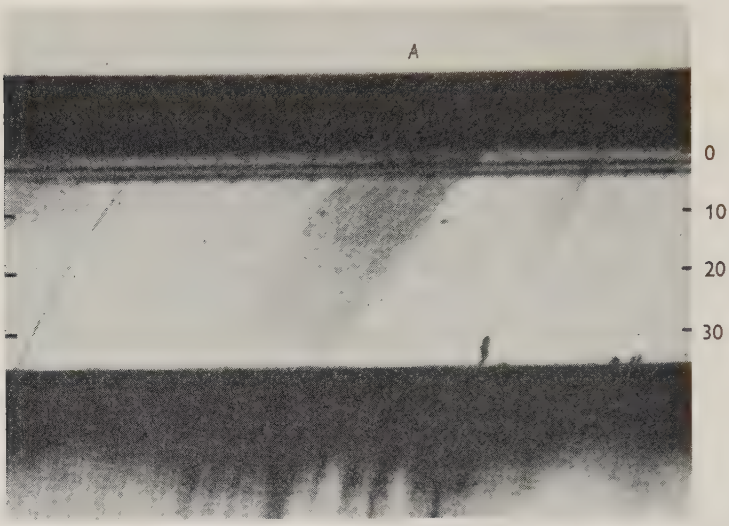

Fig. 1. The 'noisy' trace at night in Windermere. Depth in metres shown on right. The diffuse shadow at A, which appeared when the gain control was turned up, is the noisy trace.

TABLE I. ABUNDANCE AT NIGHT OF *DIAPTOMUS GRACILIS* SARS., AND *DAPHNIA HYALINA* LEYDIG

(Expressed as number per 5 l. of water at Station RNB 5.)

Depth (m)	*Diaptomus gracilis*	*Daphnia hyalina*	Total
0	131	1	132
5	47	21	68
12	31	2	33
16	6	3	9
25	—	2	2

When the diffuse trace was observed, water samples were immediately taken with a 5 l. Rodhe bottle at 0, 5, 12, 16 and 25 m. The depths were chosen according to the appearance of the trace, the record having faded at 16 m. The counts of plankton animals are given in Table I.

During the daytime, samples were taken chiefly from the depths of the thermocline, at about 10 m. When the animals were concentrated near the thermocline, their numbers reached 28/l. and a trace was recorded at that depth. When the animals were not concentrated at the thermocline, their numbers at 0, 5, 10 and 12 m were not greater than 2 or 4/l.

There are three points of evidence associating this trace with the presence of plankton animals. First, the fact that it appeared only at night, when the animals had migrated to the surface. Secondly, the trace faded at 16 m in depth, which is the lower limit to the distribution in depth of the animals; however, if the animals were uniformly distributed, the trace would be of this character because of the dissipation of transmitted energy with depth and the fading at 16 m could be a coincidence. Lastly, the density of animals that presumably produced the trace, when diffusely spread at the surface, was the same as the density that produced an echo when packed against the thermocline at 10 m, i.e. 28/l. (Cushing *et al.* in press).

RESULTS FROM THE NORTH SEA

In May and June 1953 two gears were being tested, in the North Sea, off North Shields. The first was an echo-sounder, with oscillators mounted on the keel to transmit horizontally. The second was a high-speed plankton net, which was being towed just below the surface.

The horizontal echo-sounder—pulse length, 1 ms (millisecond)—which was a development project of Messrs Kelvin and Hughes Ltd., had two ranges, 150 and 1500 fm (274 and 2742 m); the gain control of the amplifier could be conveniently divided into six points, dividing the degree of amplification into seven levels, 0, $\frac{1}{6}$, $\frac{1}{3}$, $\frac{1}{2}$, $\frac{2}{3}$, $\frac{5}{6}$ and full. The cruise was primarily a survey with an ordinary vertically transmitting echo-sounder; at stations, 10 miles apart, plankton nets were hauled from the bottom to the surface.

At each station, the following procedure was adopted for very roughly estimating the noise and reverberation level. The amplifier was set at 0, $\frac{1}{6}$, $\frac{1}{3}$, $\frac{1}{2}$, $\frac{2}{3}$, $\frac{5}{6}$ and full, successively and at each setting, the machine was allowed to make a few transmissions on the 150 fm range, when the ship was hove to on station. At each level of amplification, the paper record was marked to a certain distance in mm from the transmission mark; these distances were taken as indices of noise and reverberation. Each record was marked for measurement, when wet, so that there would then be no question of differential fading on different records. A typical record is shown in Fig. 2, giving three separate series of measurements, at 150 fm range with the ship stopped, at 1500 fm range with the ship stopped, and again at 150 fm range, when the ship was under way. Thus each record gives a measure of the noise in mm, when the sounder is transmitting horizontally beneath the sea surface.

The plankton at the surface was estimated with the use of the tin tow-net, or high-speed tow-net, hauled just below the surface for 10 min as the ship steamed away from the station. This model (described by Gehringer, 1952) stands 7 ft (213 cm.) high, has a mouth opening of 40 cm in diameter, and has a metal net of 40 meshes to the linear inch. It was towed from a bracket on its dorsal surface with a light Larsson trawl warp from the boom; a Larsson depressor was slung underneath to stabilize it. Used in this way, it was exceedingly stable at all speeds.

The net was shot from the quarter as the ship steamed away from a station; as the ship accelerated, a little warp was let out until the net was visible between 5 fm (9·1 m) and the surface. After 10 min the net was hauled in at full speed with no difficulty; the catch was washed out with a hose, bottled and preserved.

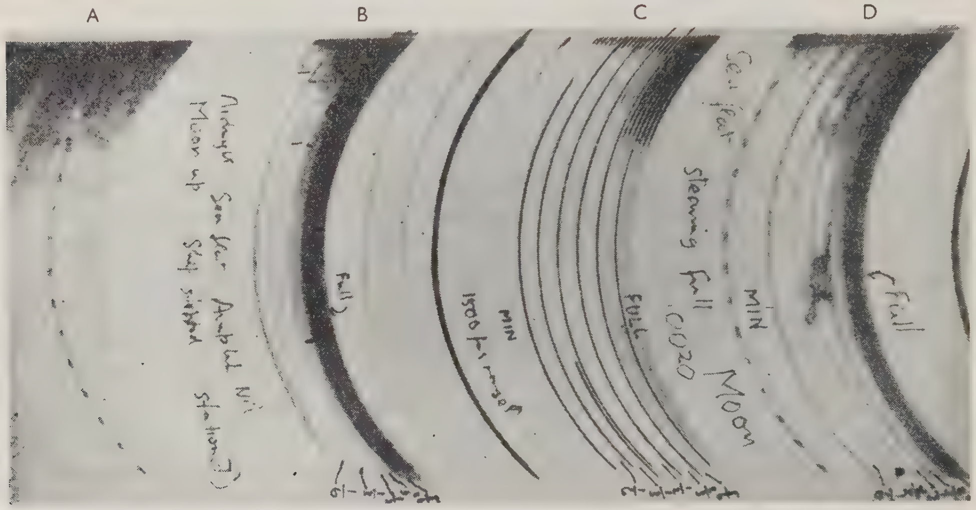

Fig. 2. An illustration of the methods used in estimating the noise levels on *Platessa* Cruise VIII, 1953. A: part of the horizontal sounder trace, as the ship approached station 7, amplifier setting $\frac{1}{3}$. B: transmisson records made at various amplifier settings, using the 150 fm range scale. C: transmission records made at various amplifier settings, using 1500 fm range scale. D: transmission records made at various amplifier settings, using the 150 fm range scale, as *Platessa* was steaming away from the station. The echo at about 80 fm in section D is from the ship's wake.

The plankton animals caught were identified as copepods, sagittae, euphausiids (*Nyctiphanes couchii* Bell), and fish larvae, and were expressed as numbers per 10 min haul; the numbers of animals were multiplied by the cubes of their lengths to give an estimate of volume. A long series of measurements of the lengths of adults and copepodite stages of *Calanus finmarchicus* Gunner, *Pseudocalanus elongatus* Boeck, *Paracalanus parvus* Claus, and *Temora longicornis* Müller, was used for this purpose (Cushing, in press). Additional length measurements of *Acartia*, *Oithona*, *Evadne*, *Sagitta*, euphausiid furcilia, and smaller larvae were also made. The results were expressed as volumes of total zooplankton in mm^3 and as volumes of euphausiids in mm^3.

Six observations were made on *Platessa* Cruise VIII 1953, during flat calm weather at night and during the day. Fifteen observations were made on *Platessa* Cruise IX 1953, under a variety of conditions. The summarized results are set out in Table II, showing volumes of zooplankton in mm^3 and the indices of noise in mm; the state of the sea is also recorded. On Cruise VIII, the degree of amplification was measured as described above, but on Cruise IX, it was placed at a constant low setting, which was unrecorded.

TABLE II. VOLUMES OF ZOOPLANKTON, IN MM^3, PER 10 MIN HAUL AND INDICES OF NOISE, IN MM

Platessa Cruise VIII, 1953

Station	Zooplankton (mm$^3 \times 10^6$)	Euphausiids (mm$^3 \times 10^6$)	Noise, in mm gain $\frac{2}{3}$ 1500 fm range	State of sea
8	1·82	0·02	6	0
6	7·35	1·75	27	0
7	4·55	3·13	21	0
30	1·90	0·07	3·5	0
8*a*	0·41	0·002	5	0
14	0·33	0·02	5	0

Platessa Cruise IX, 1953

Station	Zooplankton (mm$^3 \times 10^6$)	Euphausiids (mm$^3 \times 10^6$)	Noise in mm	State of sea
50	0·81	0·002	6·5–7	0
50*a*	0·003	0·0	5–5·5	0
51	0·68	0·001	7–7·5	0
51*a*	1·10	0·01	5·5	0
52*a*	0·80	0·002	7–7·5	2
54	0·02	0·0	5–5·5	0
54*a*	1·47	0·04	2·5–3	1–2
55	1·25	0·07	1·5	0
56	1·09	0·01	4·5	0
61	1·71	0·10	4·5–5	0
62	0·60	0·17	4	0
63	0·21	0·12	6·5	2
64	1·03	0·01	5·5–6	3
65	1·30	0·02	5·5–6	3–4
66	0·08	0·001	6·5	3–4

On Cruise VIII the highest noise indices occurred when euphausiids were present in large numbers, dominating the whole plankton catch. On Cruise IX, when the euphausiids, in general, comprised only a small part of the plankton catch, the noise indices were always about the same, there being no violent differences as there were on the previous cruise; in fact, the noise did not increase noticeably when the state of sea increased. Again the average volume of euphausiids on Cruise VIII was about twenty times that found on Cruise IX.

Fig. 3 illustrates the traces at stations 3 and 6 on Cruise VIII. Fig. 3A shows the clear trace at station 3 on the 150 fm range, with the amplifier set at $\frac{1}{3}$. Fig. 3B shows the trace at station 6 on the 150 fm range, with the amplifier set at $\frac{1}{3}$. The remarkable difference is attributed to a marked difference in quantity of zooplankton, in this case, mainly euphausiids.

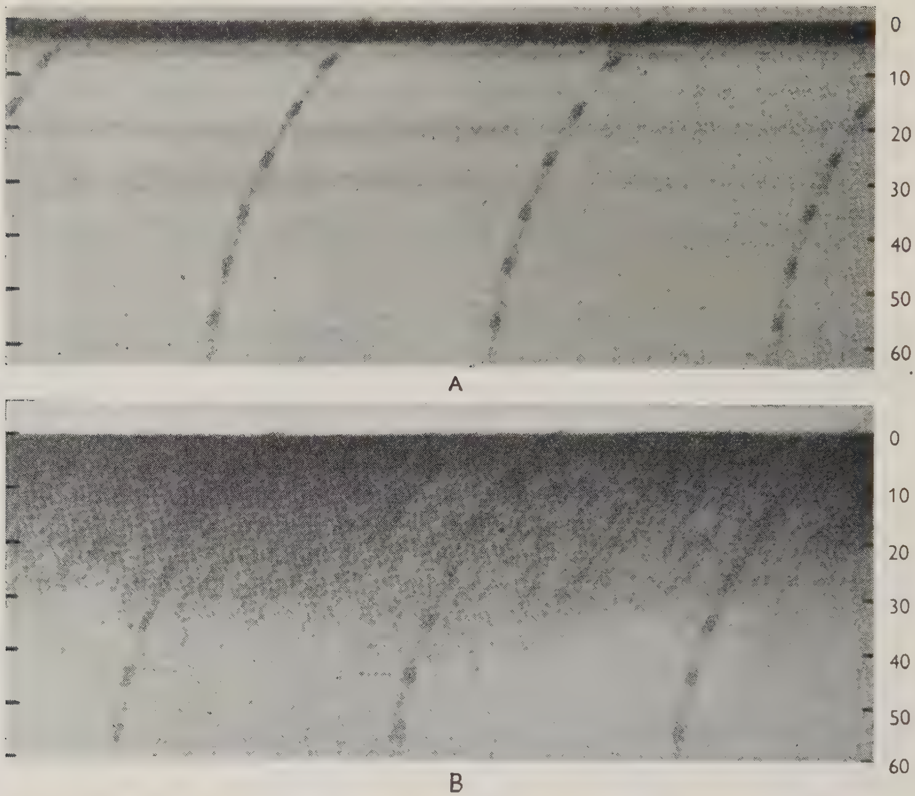

Fig. 3. The 'noisy' trace at night in the North Sea. Range in fathoms shown on right. A: part of the horizontal sounder trace observed 30 miles away from the plankton patches, in full daylight, at an amplifier setting of $\frac{1}{3}$. B: part of the horizontal sounder trace observed in the plankton patch at night, at an amplifier setting of $\frac{1}{3}$. Note the constant decrease in signal strength from the transmission outwards, with increasing range.

Discussion

The traces shown in Fig. 1 and in Fig. 3 are of a particular type. To describe it we must distinguish between noise and an echo. If an echo-sounder is switched on, but is not transmitting, the receiver is open to receive any sound of the frequency to which it is tuned; this is water noise. Again if the echo-sounder is transmitting, part of the transmitted energy is scattered by small particles, by irregularities in the temperature structure of the water and by irregularities on the bottom; some of this randomly scattered energy reaches the receiver as noise. This reverberation, as the scattering is sometimes called, is like the water noise in that it is random in character and appears as a record that is dirty all over.

Now, when the amplifier of an echo-sounder is turned up progressively, the paper becomes marked irregularly all over, with water noise, reverberation and lastly with instrument noise. An echo is distinguished from noise by its consistent appearance at about the same depth or range in successive transmissions. It is only the successive transmissions that establish the identity of an echo, as opposed to noise. This is why a paper recorder is necessary for establishing the presence of signals from fish shoals on a CRT, because, on the screen, noise and echoes cannot readily be compared with signals of previous transmissions.

The traces shown in Figs. 1 and 3 have three characters: first they are horizontally extensive, secondly they are irregularly diffuse, and lastly, when the amplifier is turned up, they show increased range. If the amplifier was turned up whilst a fish trace was being observed, the trace darkened with increased intensity of received signal, but did not increase its apparent range. Again, a fish trace nearly always has a discrete character and is rarely diffuse (however, traces of herring at Sandettié sometimes appear somewhat diffuse at night).

When the traces of euphausiids in the North Sea are compared with those of copepods and cladocerans in Windermere, it is found that the quantity of material was not so very different. At stations 6 and 7 on Cruise VIII, there were between 1·75 and $3{\cdot}13 \times 10^6$ mm^3 of euphausiids per 10 min haul with the tin townet. If we assume that the net filters 233 m^3 in 10 min when towed at an average speed of 6 knots, then the comparable quantity of zooplankton in Windermere would have amounted to $6{\cdot}5 \times 10^6$ mm^3 per 233 m^3. Here we have assumed that each copepod and cladoceran (some of which were small juveniles) occupied an average volume of 1 mm^3 (the length of *Diatopmus gracilis* was 1·128 mm, and that of adult of *Daphnia hyalina* was 1·733 mm—the more common juveniles were 1·2 mm; each value is the mean of fifty measurements). In the North Sea and in Windermere, the average plankton volume was 10 mm^3/l. in the former and 28 mm^3/l. in the latter.

Another similarity between the two traces is that both are continuous from the transmission mark outwards. This is not in itself unusual, but the only other diffuse and extensive trace, the shallow scattering layer, composed of fish larvae, appears to stay at night far enough from the surface, to allow a clear gap to appear between the transmission mark and the trace itself. The density of pilchard larvae in the shallow scattering layer in the Western Channel amounted to 0·11/m^3 as caught (or between 0·5 and 1/m^3 allowing for escape from the Petersen young-fish trawl: J. P. Bridger, private communication). If each fish larva was 8–12 mm long, there would be about 1 mm^3/l. The difference of 10 to 28 times between this estimate and that for the noisy trace is attributed to the presence of air bladders (which were perhaps resonating) in the pilchard larvae, which would scatter sound much more efficiently than would plankton animals or bladderless fish larvae.

When working in Windermere we were primarily interested in the thin echo layer associated with the thermocline. From our observations we associated the echo layer with the presence of plankton animals (*Diaptomus gracilis* and *Daphnia hyalina*) packed against it (Cushing *et al.*, in press). When the echo layer occurred at 10 m, the signal was rather weak but quite discernible, and there were 28 animals/l., which was the same density as that found by night at the surface in Windermere. Between the two signals there was probably not a great deal of difference in strength. The transmitted signal at the depth of the plankton animals was reduced by many times, through being transmitted through 10 m of water; hence, to produce a signal on the echo-sounder at all comparable, the animals must have been packed considerably more tightly than appears from the estimate of 28/l. Since our samples were taken with a Rodhe bottle about $\frac{1}{2}$ m long, one of them could easily be composed of a thin layer of animals at a density of 140/l.

There are three types of echo trace that are perhaps associated with plankton: first, the shallow scattering layer of fish larvae; secondly, the echo layer at the thermocline; and thirdly, the noisy trace. The fourth type of record that has been associated with plankton is that from the deep scattering layer, which has been attributed partly to the presence of euphausiids. The noisy trace that was found in the North Sea is interesting from this point of view, in that it was possibly composed of euphausiids.

Before we discuss the noisy trace in relation to the deep scattering layer, it would be well to consider the numbers of euphausiids caught at Stations 6 and 7 on *Platessa* Cruise VIII, 1953. To obtain a minimum estimate, it will be assumed that the tin townet filtered the water efficiently. The numbers of adult euphausiids caught in 10 min at Stations 6 and 7 were 15,900 and 28,500 respectively, which makes 68 and 122/m^3, if an average speed of 6 knots was maintained during the haul. If the net was only working at 20% efficiency, these figures must be raised by five times. Other reported catches are:

(1) Mackintosh (1934). The average numbers of *Euphausia superba* per haul is 1,000, which is 1–5/m^3, assuming 20% efficiency. The highest catch was 190–950/m^3.

(2) Hardy & Gunther (1935), figs. 90, 92, 95, table 52. The highest numbers of *Euphausia frigida*, *E. superba* and *Thysanoëssa* spp. were up to 150/m^3, assuming 20% efficiency.

(3) Einarsson (1945). Assuming 20% efficiency, the highest catch of *Thysanoëssa longicaudata* was 4–12·5/m^3.

(4) Moore (1950). *Euphausia brevis*. 1·5/m^3; by inference from luminescence observations at night, 7–15/m^3.

(5) Boden (1950). *E. pacifica*; assuming 20% efficiency, the highest catch was 2·5/m^3.

(6) Glover (1952). *E. krohnii*, *Meganyctiphanes norvegica*, *Nyctiphanes couchii*, *Thysanoëssa inermis*, *T. longicaudata*; assuming perfect filtration with the plankton recorder, there were 1–3/m^3 (or 5–15/m^3, assuming 20% efficiency).

It follows that the catches made in the North Sea in May were amongst the highest recorded. This might have been associated in part with the moon-light drawing the euphausiids to the surface, but it is also believed that an important factor was the use of the high-speed tin townet, which has a wide enough mouth to make escape at a towing speed of 6–8 knots (309–412 cm/sec) nearly impossible.

During these trials, euphausiids were found at a density in numbers of perhaps 120/m^3, yet signals in the noisy trace were not recorded from beyond 30–80 fm (55–146 m) range; the nature of the trace and the character of our observations, continuously for 3 h over 20 miles of sea, do not lead us to suspect that there was a boundary to euphausiid distribution running parallel to the ship's course at a range of 80 fm (146 m).

In our North Sea observations euphausiids did not give a signal beyond 80 fm (146 m) depth, whereas the deep scattering layer was recorded down to 450 fm (823 m). There is evidently a difference here of a large order of magnitude, which might be either instrumental or biological. It is difficult to believe that our machine was very much less sensitive than those used in studies on the deep scattering layer because the relatively high figure of 1 kw was put into the transducer although the pulse length was short; so the signal-to-noise ratio was lower than if a longer pulse had been used. To our minds, our observations present yet another reason for doubting that that layer is due to euphausiids.

SUMMARY

An echo trace attributable to plankton organisms has been described. Its diagnostic character is that when the amplifier of the echo-sounder is turned up, the trace extends in range; a fish trace would not extend in range but would darken with increased intensity of signal.

This trace was observed at night in Windermere where it was possibly composed of copepods and cladocerans at a density of about 28 animals/l.; it was also observed at night in the North Sea, where it was probably composed of euphausiids at a density of 0·12 animals/l.

The euphausiids were caught with a high-speed townet, with a mouth opening of 40 cm diameter. A very rough comparison was made between the North Sea trace of euphausiids and the signal that comes from the deep scattering layer, which may be partly composed of euphausiids.

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FILTERING, FEEDING, AND DIGESTION IN THE LAMELLIBRANCH *LASAEA RUBRA*

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(Text-figs. 1–11)

Lasaea rubra is the smallest and commonest of Plymouth bivalves. It is intertidal on rocky shores, often in immense numbers, in crevices and other protected places, and in *Pygmaea pumila* (Colman, 1940). This bivalve is an excellent laboratory animal. It remains fully active for several days under laboratory conditions, and a large number of animals can be used in a single experiment, eliminating the variations in activity found when dealing with a few, or single specimens of larger animals. Because of the relatively high position *Lasaea rubra* may occupy on the shore its feeding cycle is broken by regular dry periods at each tide. Thus, for experiments relating to periodicity in feeding, we have an animal whose times of feeding can easily be ascertained and experimentally varied over a wide range. Lastly, in making serial sections and in examining total gut contents, in work on the digestive system, the small size of the animal is of obvious advantage.

The present paper is devoted to an account of filtering rates with various food organisms, variations in filtering activity and the relation of filtering to feeding and digestion. Further work has been completed by one of us (J. E. M.) on the mechanism of digestion and the cycle of activity of the digestive gland, and experiments are in progress on respiration during submersion and exposure.

We record our special thanks to Dr Mary Parke, botanist at the Plymouth Laboratory, for making available the unialgal cultures of the organisms used, and for her invariable kindness and sound advice. One of us (J. E. M.) has been the holder of a University of London Table at the Plymouth Laboratory, and both of us are grateful to the Director and staff of the Laboratory for many kindnesses. In particular, Dr H. W. Harvey, F.R.S., allowed us the frequent use of his absorptiometer, and gave us great encouragement throughout the work, while Dr B. C. Abbott gave valued assistance in discussion and evaluation of results.

METHODS

Specimens of *Lasaea rubra* were collected on the day of each experiment from the limestone rocks below the Hoe bathing pool, in front of the Plymouth Laboratory. To ensure the sampling of a constant population, collections were made, except where otherwise stated, from as nearly as possible the same group of crevices on an outcrop of rocks 18 in. to 2 ft. below the upper limit of *Chthamalus stellatus*. The animals were used as soon as possible after collection. In experiments on filtering rates a group of twenty animals was selected for each experiment and placed in cultures of micro-organisms, in optical glass cuvettes supplied by the Tintometer Co. Ltd., Salisbury. Six such vessels were used in sets of replicate experiments, each containing 3·5 ml., with an optical depth of 20 mm. The unialgal cultures employed as food were grown in Erdschreiber (enriched sea water) medium, and it was found that *Lasaea* rapidly resume normal behaviour in this medium, aggregating in a cluster at the bottom of the cuvette and attaching to each other by byssus threads, with foot and siphon extended (Fig. 1). At this stage normal filtering activity was assumed to have begun. With practice in selection, the eye could pick out samples of *Lasaea rubra* conforming closely to a standard size distribution (Fig. 2A).

Filtering rates were determined by the measurement of the optical density of the medium by means of a Harvey absorptiometer, constructed as described by Harvey (1948). The chief modifications introduced were those outlined by Spencer (1954) for use with micro-organisms. After a series of density-dilution comparisons (see Fig. 2B) the concentration of organisms was taken to be directly proportional to log optical density. Experiments were continued for periods of 6–8 h, and readings of the optical density of the cultures were taken at intervals. An adaptation period of the first hour was allowed, and the rate of filtration was then calculated from the first to the fourth hours. In the experiments using *Arenicola* haemoglobin, measurements were made on the Unicam spectrophotometer.

In experiments where filtering, feeding and digestion were compared it was not possible to allow the animals to adapt themselves to experimental conditions for the first hour. In these experiments measurements were made of the amount of *Phaeodactylum tricornutum* filtered, the amount extruded as pseudofaeces, the amount ingested and the amount digested. In order to do this four experiments were set up and one was discontinued every half hour. After the period of filtration, optical density was measured in order to calculate the quantity of *Phaeodactylum* filtered, 3 ml. of the supernatant culture was then removed, and the remaining 0·5 ml. containing the pseudofaeces was then brought up to the original volume by the addition of 3 ml. of filtered sea water. A second reading of optical density was then made, which—after correction for the 0·5 ml. of supernatant culture left—gave the quantity of

Phaeodactylum in the pseudofaeces. In each experiment the difference between the amount filtered and the amount recovered as pseudofaeces was taken to represent the fraction removed by ingestion. This difference was found in practice to be too small to measure accurately by the absorptiometric method. Gut contents were therefore measured directly by the quick dissection of two

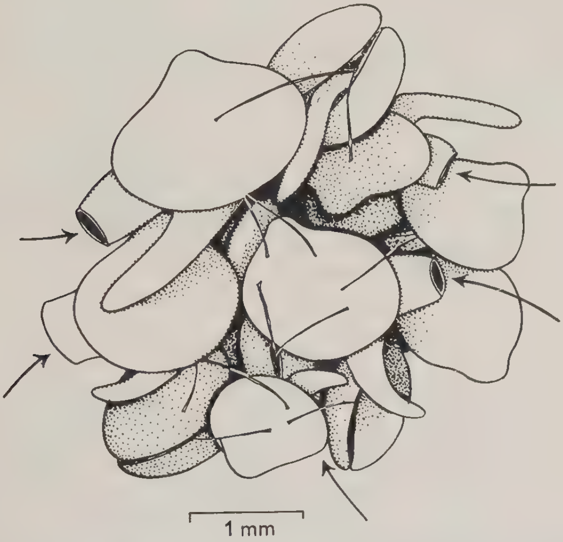

Fig. 1. A group of *Lasaea rubra*, attached together by byssus threads in the normal manner. The inhalant siphon and the foot are extended, and the arrows show the directions of inhalant water currents.

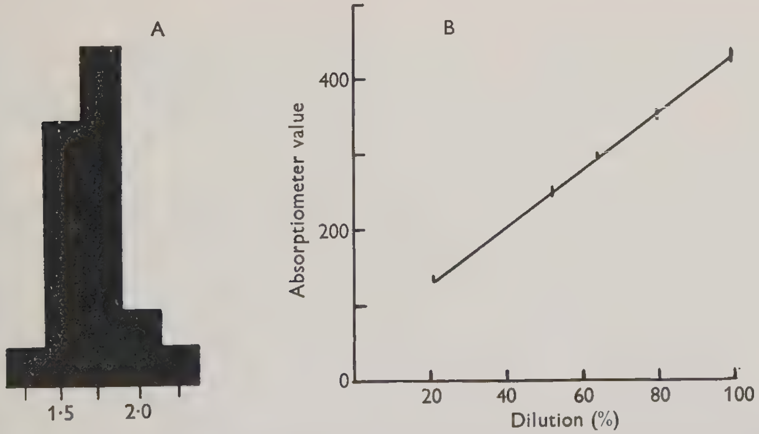

Fig. 2. A: size distribution in mm of a typical group of 20 *Lasaea rubra*, selected in experiments on filtering rates. B: the relation between dilutions of a culture of *Phaeodactylum tricornutum* and the scale of optical density on the Harvey absorptiometer.

animals from each cuvette, on dry slides under a binocular microscope. The full stomach, and as much of the intestine as contained faeces was isolated and opened, so that the total contents were shed upon the slide. Remains of tissue were as far as possible removed and the total gut contents taken up in 0·2 ml. of filtered sea water. The concentration of *Phaeodactylum* cells was then estimated by haemacytometer counts, a measure being thus obtained of the total number of organisms ingested, and of the proportion of intact, undigested cells, and of 'ghosts' constituted by the thin walls of empty, digested cells. (The proportion of 'ghosts' in the original culture was found for present purposes to be negligible.)

In experiments using mixed suspensions the proportions of the various constituents of the suspension were measured by haemacytometer counts before the experiments and either the supernatant culture, pseudofaeces or stomach contents were again counted at the end, in order to determine whether any selection in feeding had occurred.

RESULTS

In the series of experiments on filtering rates a large number of results were obtained with *Phaeodactylum tricornutum* (Table I). *Phaeodactylum* was selected for this purpose because it was both readily available in culture and acceptable to *Lasaea*. After the first hour filtering was usually continued at a more or less constant rate for 6 or 7 h, and Fig. 3 shows curves for a typical set of six experiments. Filtering rates were calculated from optical density by applying the formula

$$P_t = P_0 \exp\left[-\frac{m}{M}t\right],$$

used by Jørgensen (1949).[1] Initial optical density at 1 h was corrected to 500, and the fall in 3 h was calculated from the corrected 4 h reading, using 3·5 ml. of culture and a group of 20 animals in each cuvette. Thus, *m*, equalling the volume in ml. of water filtered by 20 animals in 1 h, was calculated as 2·684 ($2{\cdot}699 - \log_{10}$ corrected optical density after 3 h).

The effects of temperature on the filtering rate with *Phaeodactylum* are expressed in Fig. 4. It may be considered that temperature changes will exert an effect on the direct rate of current flow (see Gray, 1928) rather than on the efficiency of filtration by the gill; but Fig. 4, which is closely comparable with Galtsoff (1928, p. 23, fig. 8), suggests that the measurement of filtration rate gives a reliable measure of current flow. The scatter is widest in the experiments at the lower temperature range, where the behaviour of the bivalves was least predictable.

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[1] M = quantity of water in cuvette; m = quantity of water filtered; P_t and P_0 are the concentrations of suspended material at times t and 0 respectively.

TABLE I. FILTERING RATES OF *LASAEA RUBRA* WITH *PHAEODACTYLUM TRICORNUTUM*

(Each value represents the volume filtered in ml./h, over 3 h by 20 animals, at room temperature.)

Set of experiments	Filtering rates for 20 animals
1	0·403, 0·681, 0·513, 0·786
2	0·9162, 0·2203, 0·6764, 0·8758, 0·450, 0·952, 0·543, 0·471, 0·550, 0·339, 0·963, 0·698, 0·380, 0·311, 0·212, 0·339, 0·260, 0·499, 0·189
3	0·565, 0·565, 0·963, 0·612, 0·680, 0·754, 0·456, 0·745, 0·804, 0·499, 0·492, 0·488, 0·715, 0·664, 0·644, 0·506, 0·496, 0·715, 0·685, 0·676
4	0·364, 0·308, 0·419
5	0·406, 0·536, 0·399
6	0·4744, 0·9212, 0·809, 1·435, 0·628, 0·847
7	0·795, 0·754, 0·524, 0·577, 1·469, 0·8179
8	0·3672, 0·6396, 0·9162

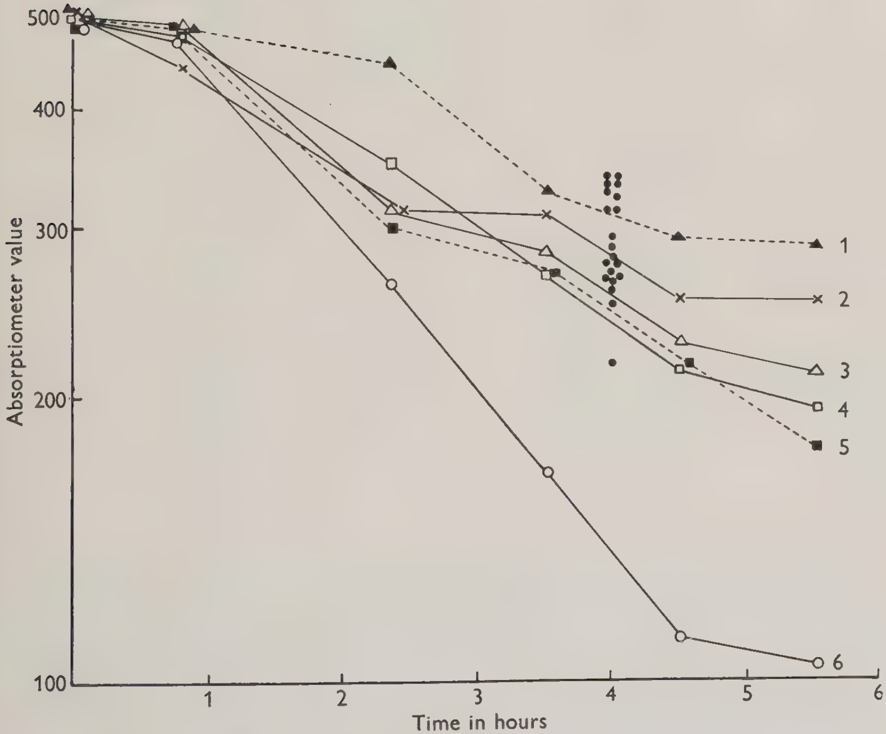

Fig. 3. Six curves showing the filtering rates of sets of 20 *Lasaea rubra* in *Phaeodactylum*, in a typical series of experiments over $5\frac{1}{2}$ h. It is suggested that the highest rate (Expt. 6) approaches the value likely to be achieved by continuous filtering by all the animals during the whole experimental period. The scatter points show the end values of optical density in a further series of twenty experiments, all values being determined with reference to an initial base line of 500 absorptiometer units.

Comparison with other Lamellibranchs

A pumping rate of 1 ml./h/20 animals at 15° C is cited in Table II for *Lasaea rubra* as being typical of the higher rates obtained with groups of mainly adult animals, sampled as in Fig. 2A. Table II summarizes the information on the filtering rates for other lamellibranchs. Verwey (1952, pp. 193–4) gives a useful review of what has already been published on this subject. Filtering rates are produced by comparison of the volume of water filtered by one animal with the volume by displacement of the total animal with shell closed.

TABLE II. COMPARISON OF THE FILTERING RATE OF *LASAEA RUBRA* WITH OTHER LAMELLIBRANCHS

Species	Author	Rate of filtering (ml/h)	Estimated mean volume of single animal	Vol. water filtered in 1 h/volume of animal	Temp (° C
Lasaea rubra (sample as in Fig. 2A)	—	1 ml. approx. (for 20 animals)	1·2 mm³	42	15–1
		By direct method			
Ostrea virginica	Loosanoff & Nomejko (1946)	13,000 to 20,000	100 cm³	130–200	—
O. virginica	Galtsoff *et al.* (1947)	6,000 to 12,000	100 cm³	60–120	—
O. virginica	Galtsoff (1928)	3,900 (max.)	100 cm³	39	—
		Immature and growing animals—indirect method			
Mytilus edulis (15 mm)	Jørgensen (1949)	160	0·8 cm³	200	17–2
M. edulis (30 mm)	Jørgensen (1949)	750	5 cm³	150	17–2
		Mature animals—indirect method			
M. edulis (48 mm)	Willemsen (1952)	1,100	19 cm³	58	12–1
M. edulis (67–69 mm)	Willemsen (1952)	1,700	30 cm³	57	12–1
M. edulis (77–80 mm)	Willemsen (1952)	1,900	40 cm³	47	12–1
M. californianus (74 mm)	Fox, Sverdrup & Cunningham (1937)	1,400	38 cm³	35	20–2
Cardium edule (30–40 mm)	Willemsen (1952)	500	*c.* 23 cm³	22	17·3– 19·5

Filtering Rates with other Organisms

Table III gives the list of organisms employed for comparative experiments on the filtering rate of *Lasaea*. Each was presented in unialgal culture at or slightly below the concentration of the laboratory culture, and the filtering rates obtained are shown in Table IV and Fig. 5, to which the range of values for experiments with *Phaeodactylum* has also been added for comparison.

As can be seen from Table IV a striking variation appeared in the filtering rates with various organisms, in particular with *Gymnodinium veneficum*, in which no sustained filtering is observed at all, and which has been shown to be toxic to fish, other molluscs (Ballantine, unpublished) and *Hemimysis lamornae* (Bainbridge, 1953, p. 393, as *Gymnodinium* II). Experiments were therefore carried out on the effect of pre-treatment with *G. veneficum* on the subsequent filtering rate in *Phaeodactylum*. When placed in the toxic culture the *Lasaea* fail to aggregate or to put out the foot and siphon, but in spite of this shell-closure some of the toxin (which is present both in the cells and the

TABLE III. PHYTOPLANKTONIC ORGANISMS USED IN EXPERIMENTS ON FILTERING RATES OF *LASAEA RUBRA*

Plymouth culture no.	Organism	Classification	Cell size (μ)
85	*Chlorella stigmatophora* Butcher	Chlorophyceae	2·5–4·5
90	*Chromulina pusilla* Butcher	Chrysophyceae	1–2
B	*Dicrateria inornata* Parke	Chrysophyceae	3–5·5
28	*Exuviaella baltica* Lohm.	Dinophyceae	9–15
103	*Gymnodinium veneficum* nom. prov. Ballantine	Dinophyceae	9–19
102	*G. vitiligo* nom. prov. Ballantine	Dinophyceae	7–18
I	*Isochrysis galbana* Parke	Chrysophyceae	5–6
87	*Nannochloris atomus* Butcher	Chlorophyceae	2–3
104	*Peridinium trochoideum* (Stein) Lemm.	Dinophyceae	20–30
100	*Phaeodactylum tricornutum* Bohlin (=*Nitzschia closterium* f. *minutissima* of Allan & Nelson)	Chrysophyceae	20–40
97	*Prorocentrum micans* Ehr.	Dinophyceae	30–40

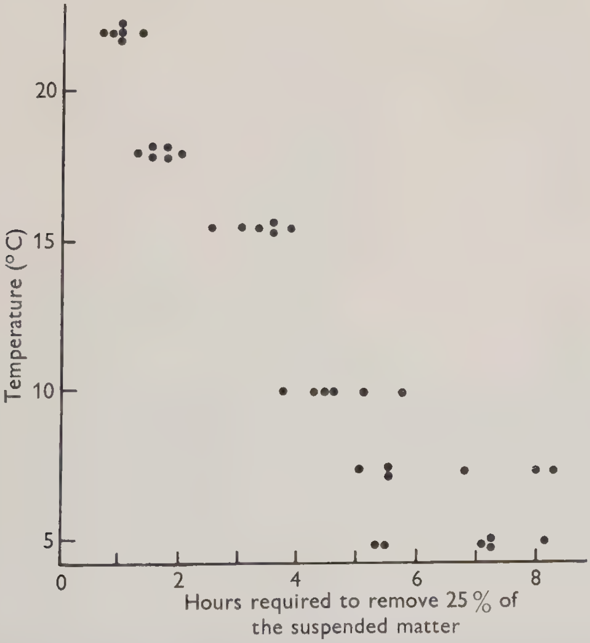

Fig. 4. The relation between filtering rate of *Lasaea* in *Phaeodactylum* and temperature. Filtering rate is expressed inversely as the time required (in hours) to remove 25 % of the suspended food under the experimental conditions. (Cf. Galtsoff, 1928, fig. 8.)

supernatant water) must enter the mantle cavity, and the extent of the retardation of subsequent filtering is roughly proportional to the duration of contact with the toxin (eventually nearly all animals appeared to recover). On removal from the toxic culture the experimental animals were washed for 30 min in several changes of filtered sea water, and full activity of foot and siphon was observed before placing them in *Phaeodactylum* culture. Control experiments were carried out with animals in sea water for a period equal to the exposure to *Gymnodinium*. Table V and Fig. 6 show the results obtained in these experiments, and Fig. 7 shows the end-points, referred to a base-line of 500 units of optical density, in two series of experiments and controls.

TABLE IV. FILTERING RATES WITH VARIOUS ORGANISMS CALCULATED OVER A 3 H PERIOD

		(Expressed as ml./h/20 animals)	
Expt. no.	Organism (see Table III)	Rates for separate experiments	Average rate, calculated from mean density decrease
1–6	*Chlorella stigmatophora*	0·481, 0·426, 0·377, 0·326, 0·263, 0·226	0·346
7–12	*Chromulina pusilla*	2·120, 1·515, 1·490, 1·490, 0·910, 0·888	1·027
13–18	*C. pusilla*	0·860, 0·840, 0·766, 0·550, 0·487, 0·403	
19–24	*Dicrateria inornata*	0·449, 0·416, 0·294, 0·246, 0·270, 0·246	0·443
25–28	*D. inornata*	0·880, 0·643, 0·502, 0·491	
29–34	*Exuviaella baltica*	1·184, 0·994, 0·870, 0·860, 0·814, 0·814	0·902
35–40	*Gymnodinium veneficum*	No filtering	—
41–46	*G. vitiligo*	0·606, 0·426, 0·374, 0·370, 0·366, 0·223	0·384
47–52	*Isochrysis galbana*	2·181, 1·562, 1·338, 1·298, 0·688, 0·595	1·171
53–55	*I. galbana*	1·150, 0·941, 0·785	
56–61	*Nannochloris atomus*	0·436, 0·374, 0·354, 0·314, 0·297, 0·296	0·343
62–67	*Peridinium trochoideum*	0·492, 0·320, 0·192, 0·139, 0·176, 0·097	0·293
68–70	*P. trochoideum*	0·492, 0·387, 0·342	
71–76	*Prorocentrum micans*	1·129, 1·129, 1·129, 1·068, 0·860, 0·524	1·079
77–81	*P. micans*	1·365, 1·359, 1·230, 1·198, 0·780	

Filtering Efficiency

In dealing with the filtering efficiency of the gill, and the problem of the presence of a mucous sheet over the gill aiding in the retention of minute particles, several lines of approach were used. In sections of *Lasaea rubra* stained with Alcian blue (see Steedman, 1950), or with mucicarmine or azan, mucous glands could not be detected in the epithelium of the filaments. This evidence is admittedly open to the objection that the contents of glands actually present might have been discharged during feeding immediately prior to fixation, but even on the gills of animals fixed while actually filtering 'Aquadag' no mucous covering could be detected by these staining methods.

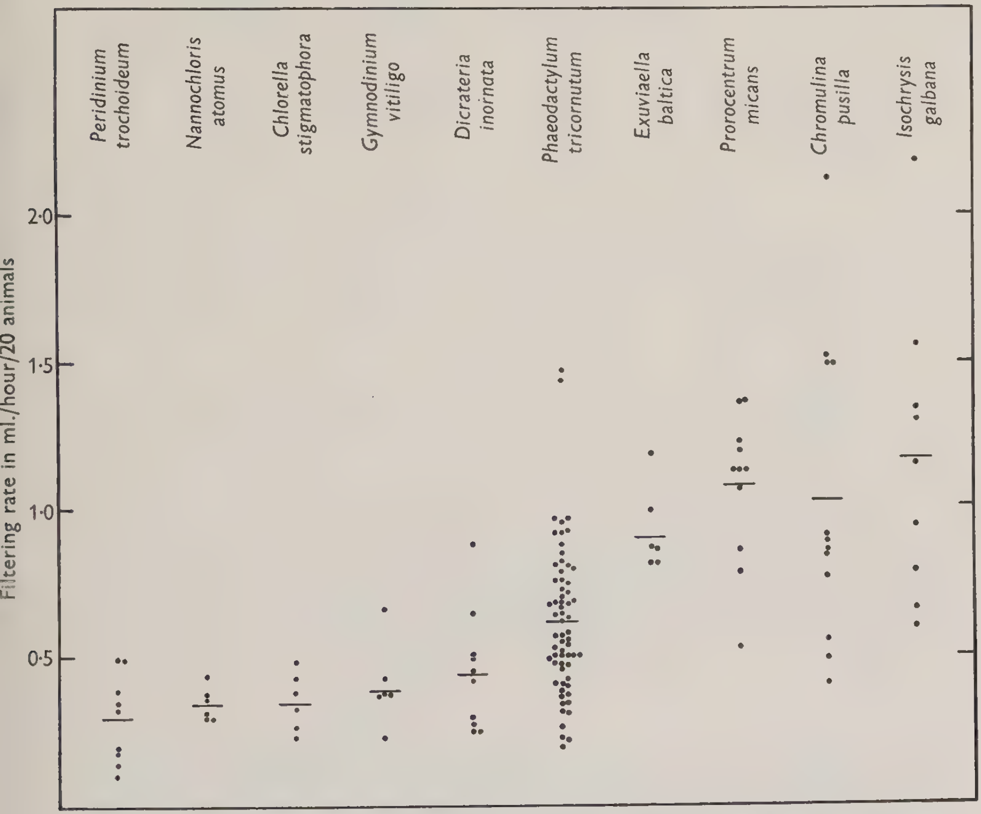

Fig. 5. The range of filtering rates exhibited by *Lasaea rubra* in all experiments with *Phaeodactylum* (Table I), and with the various organisms listed in Table III. The horizontal line on each range of values indicates the average filtering rate with that organism. *Gymnodinium veneficum* is omitted, as showing no measurable filtering rate in any experiment.

Lasaea has also been shown to filter organisms and particles of size ranging from 1 to 50 μ (i.e. from *Chromulina pusilla*, marine bacteria and 'Aquadag' to particles of 'Kieselguhr'). In general, the size of the organism, like the concentration of the suspension, appeared to have no correlatable effect on the

TABLE V. EFFECT OF PRETREATMENT WITH *GYMNODINIUM VENEFICUM*

(Filtering rates of *Lasaea rubra* in *Phaeodactylum tricornutum.*)

					Filtering rate (ml./h/20 animals)		
					Filtering rate calculated from mean density decrease	Extreme variations of filtering rate	
Group no.	Pre-treatment	No. of expts.	Mean decrease in density in 3 h	95 % confidence limits		Highest	Lowest
1	None	20	218·5	16·02	0·67	0·966	0·457
	None	23	223	28·11	0·69	1·436	0·314
	5 h in *Gymnodinium*	20	147·8	15·2	0·411	0·644	0·243
	5 h in *Gymnodinium*	20	143	27·71	0·396	0·937	0·113
	Variable time in *Gymnodinium*	18	151	28·6	0·42	0·921 (2 h in *Gymnodinium*)	0·065 (10 h in *Gymnodinium*)
2	None	19	169	31·6	0·48	0·963	0·189
	5 h in *Gymnodinium*	18	72·3	15·89	0·182	0·351	0·06
			Mean decrease in 10 h				
3	10 h in *Gymnodinium*	6	189	41·43	0·166	0·189	0·104

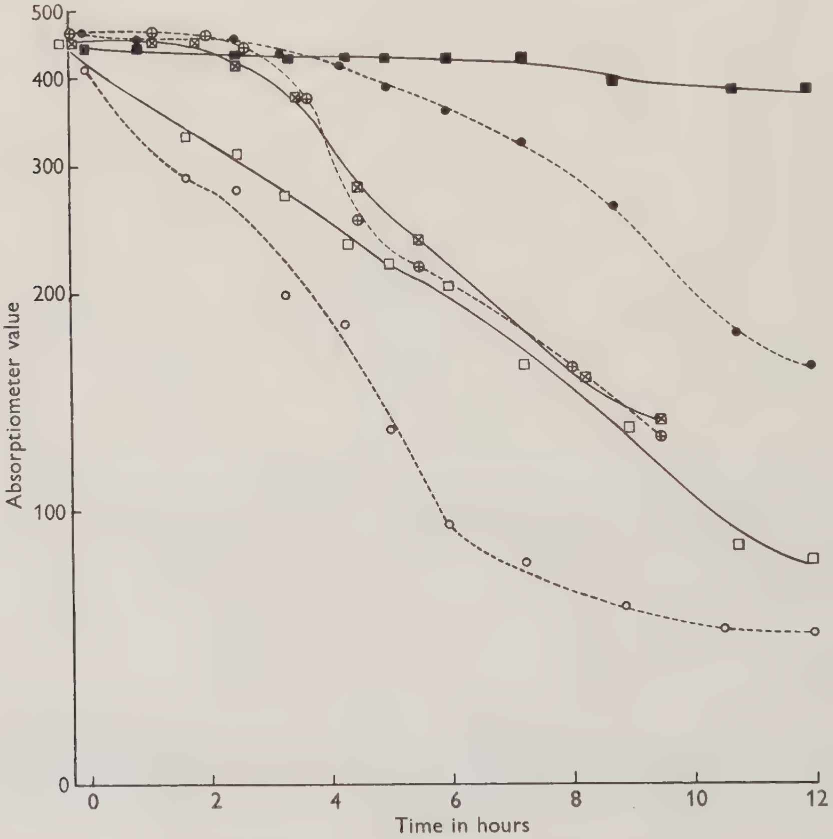

Fig. 6. The effect of pretreatment with *Gymnodinium veneficum* on the subsequent filtering rate of *Lasaea* in *Phaeodactylum*. □——□ and ○– – –○ are curves for control experiments; ⊠——⊠ and ⊕– – –⊕ for animals pretreated for 2 h in *Gymnodinium*; and ■——■ and ●– – –● for animals pretreated for 10 h.

filtration. This would indicate a high degree of filtering efficiency of particles down to 1 μ size.

With marine bacteria suspensions (Fig. 8) some filtration occurred in the first 20 min, but later ceased, probably because of lack of oxygen, and the possible toxicity of the bacteria themselves.

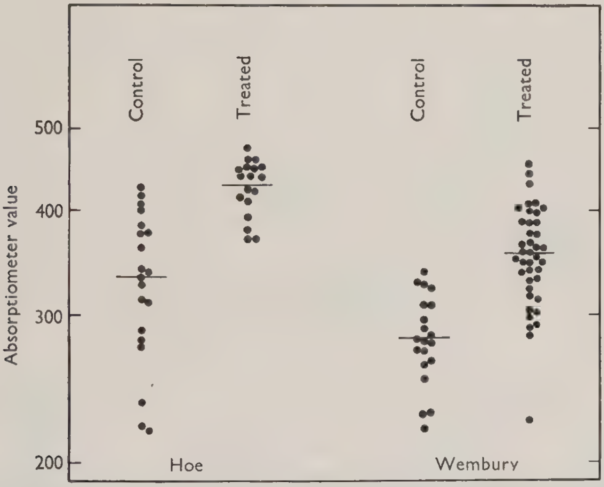

Fig. 7. The fall in optical density in 3 h of *Phaeodactylum* culture (from an initial base level of 500 absorptiometer units) in experiments using *Lasaea rubra* pretreated for 5 h with *Gymnodinium veneficum*, as compared with controls using *Lasaea rubra* from sea water. The horizontal line on each range is the average for that range. Two sets of experiments are recorded, and the higher level of the values obtained from Wembury is to be attributed to the use of animals larger than the average experimental size illustrated in Fig. 2A.

In addition to experiments using colloidal graphite ('Aquadag' 'c' and Graphite 's'), which gave rather variable results, work was carried out based on the results of Jørgensen & Goldberg (1953) on the removal from suspensions, by the gill of *Mytilus* and *Ostrea*, of large protein molecules of vertebrate haemoglobin, and *Haliotis* and crab haemocyanin. The greatest retention (25%) was obtained by Jørgensen & Goldberg with crab haemocyanin, with less than 10% retention in the other experiments. It was suggested that filtration by, or adsorption on, a mucous sheet was responsible for the uptake of these large molecules. Parallel cases are the reports of the survival of clams for periods of several months in filtered beef-tea, and the known ability of lamellibranchs to live for long periods in particle-free solutions of organic nutrients. A series of experiments was carried out on *Lasaea*, using sets of 20 animals in 5 ml. of a solution of *Arenicola marina* haemoglobin in sea water (Table VI). It is perhaps necessary to point out that the percentages quoted in these results are not directly comparable with those of Jørgensen & Goldberg, as experimental conditions varied in ways not ascertainable from the results as presented.

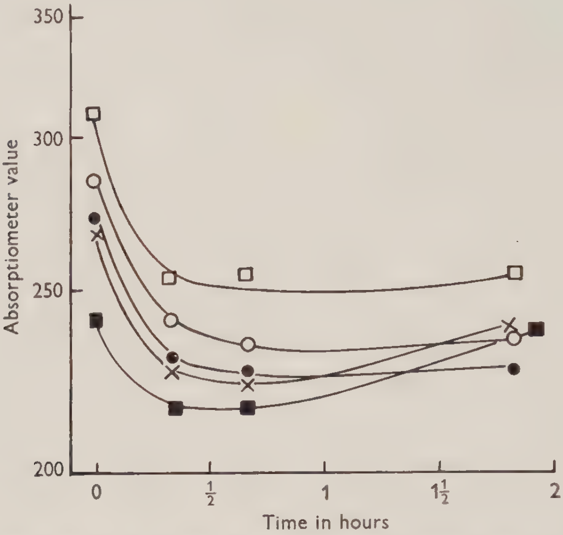

Fig. 8. The filtering activity of *Lasaea* in suspensions of marine bacteria. Filtering ceased after 40 min, and the slight increase in optical density in some of the experiments after 2 h is probably to be attributed to bacterial division and the resuspension of bacteria from released pseudofaeces.

TABLE VI. REMOVAL OF THE HAEMOGLOBIN OF THE BLOOD OF *ARENICOLA MARINA*, AFTER 12 H

		Readings at 415 mμ peak*			Readings at 540 mμ peak	
Expt. no.	1st reading	2nd reading	% fall	1st reading	2nd reading	% fall
1 } 20 *Lasaea* used in each expt.	1350	1150	15	—	—	—
2	1350	1150	15	—	—	—
3	1350	1275	5·5	210	195	9·3
4	1350	1275	5·5	210	180	8·6
5 } Controls (No animals)	1350	1350	—	210	210	—
6	1350	1360	—	210	210	—

* A slight shift of peak between the first and second readings occurred both in experiments and controls, possibly due to oxidative changes.

Filtering, Feeding and Digestion

In experiments for the comparison of filtering, feeding and digestion rates (Tables VII and VIII), *Lasaea rubra* was found to ingest *Phaeodactylum* rapidly to the full capacity of the stomach. With the use of a thick suspension, the amount required to fill the gut is only a small fraction of the total filtered.

The surplus of the filtered material is extruded from the pallial cavity as loosely compacted pseudofaeces. The results obtained using cultures of *Phaeodactylum* show that after 2 h 40% of the material within the stomach was digested, after continuous heavy feeding up to the time of examination. The results from Table XIV indicate that within a short time the total food ingested may be submitted to digestion.

Inspection of the curves for the four experiments shown (Fig. 9A) reveals that the rate of filtering continued to increase over the 2 h experimental period. Since, however, the curve for each of the four experiments closely agrees with the curve obtained by plotting the end-points of Expts. 1–4, it is considered that this artificial curve gives an accurate picture of the conditions obtaining in any of the individual experiments. The larger value of the filtering rate towards the end of the experiments (Table VII), as compared with the values cited in Table I, may be ascribed to the greater number of large-sized *Lasaea* used in order to facilitate dissection of the gut. The shape of the curves, and the progressive increase in filtering rate, is probably due to the increased delay shown by these larger animals in acclimatizing themselves and resuming natural behaviour in the cuvettes. The total of digested cells can be seen to have risen steadily throughout the experiment (Fig. 9B), and the proportion of digested cells is probably rather greater than is apparent here, as some cells must have been completely digested, and have disappeared altogether. This number is not likely, however, to be large, as with experience even the faintest 'ghost' cells can be recognized, and the total time of the experiment was not sufficient for very extensive digestion. Fig. 10 sums up the evidence from these experiments in a single diagram.

TABLE VII. SYNOPSIS OF RESULTS IN EXPERIMENT ON FILTRATION AND FEEDING IN *LASAEA RUBRA*

Time from start	Filtering rate (calculated from fall in density of supernatant) (ml./h/ 20 animals)	Total no. of *Phaeodactylum* filtered	Total no. of *Phaeodactylum* in pseudofaeces (from counts and calibration curve)	No. of *Phaeodactylum* in stomachs of 20 animals
½ h	0·815	0·77 × 10^6	0·7 × 10^6	0·0725 × 10^6
1 h	1·100	2·99 × 10^6	2·06 × 10^6	0·93 × 10^6
1½ h	1·205	6·63 × 10^6	3·67 × 10^6	2·90 × 10^6
2 h	1·825	13·39 × 10^6	8·79 × 10^6	4·60 × 10^6

Most recent opinion agrees that any sorting that occurs on the gills or labial palps of lamellibranchs takes place by size and weight of particles, and that 'selection on other grounds, as according to organic or inorganic content, or according to digestibility or unpalatability is out of the question' (Verwey, 1952). But since Fox (1936) earlier maintained that particles could be sorted according to their acceptability, and Loosanoff (1949) states that feeding *Ostrea* are capable of choosing between different flagellates, it was decided to

TABLE VIII. FEEDING AND DIGESTION IN A CULTURE OF *PHAEODACTYLUM*

(Starting time of experiment 10.00 h, 18 August 1954.)

Total *Phaeodactylum* in stomach in thousands

		Animal 1			Animal 2			Average		
Expt. no.	Time	Intact cells	Digested 'ghosts'	Total	Intact cells	Digested 'ghosts'	Total	Intact cells	Digested 'ghosts'	Total
1	10.30	4	—	4	3	0·25	3·25	3·5	0·12	3·62
2	11·00	37	8	45	28	10	38	32·5	9	41·5
3	11.30	82	40	122	134	40	174	108	40	148
4	12.00	102	80	182	172	106	278	137	93	230
5*	12.30	16	7·5	23·5	24	8	32	20	7·75	27·75

* The rate of Expt. 5 was noticeably slower than that of the other 4; the values at 12.30 h approximate most closely to those of Expt. 2 at 11.00 h. These figures (Expt. 5) have not been expressed in the graphs in Fig. 9.

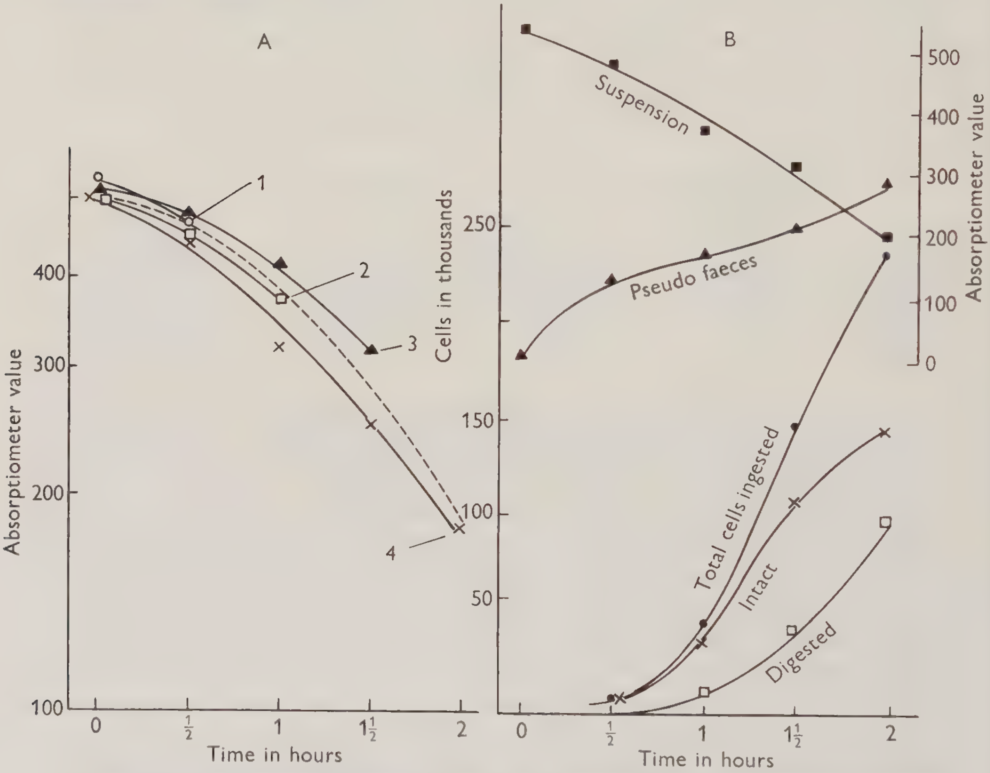

Fig. 9. A: the filtering rate of *Lasaea* in *Phaeodactylum* over 2 h in Expts. 1–4 referred to on p. 252. The broken line represents the hypothetical curve obtained by joining the points at which Expts. 1–4 were discontinued. B: above—the optical density of the suspension of *Phaeodactylum* and of the resuspended pseudofaeces, over 2 h, in the experiments referred to on p. 252; below—the numbers of cells (total, intact and digested) in the stomach of a single *Lasaea* at the same intervals (numbers estimated by haemacytometer counts).

carry out some experiments on discrimination in *Lasaea*. It seemed likely that, in a small lamellibranch able to digest naked or thin-walled organisms better than diatoms and or armoured dinoflagellates, any sorting mechanisms available for rejecting large or unsuitable organisms would operate very effectively. Experiments were set up with the following mixtures, each cuvette containing 20 *Lasaea*: (*a*) *Phaeodactylum tricornutum* / *Dicrateria inornata*; (*b*) *Peridinium trochoideum* / *Dicrateria*; (*c*) *Phaeodactylum* / *Chromulina pusilla*; (*d*) *Phaeodactylum* / *Thalassiosira fluviatilis*; (*e*) *Phaeodactylum* / 'Kieselguhr' (diatomaceous earth).

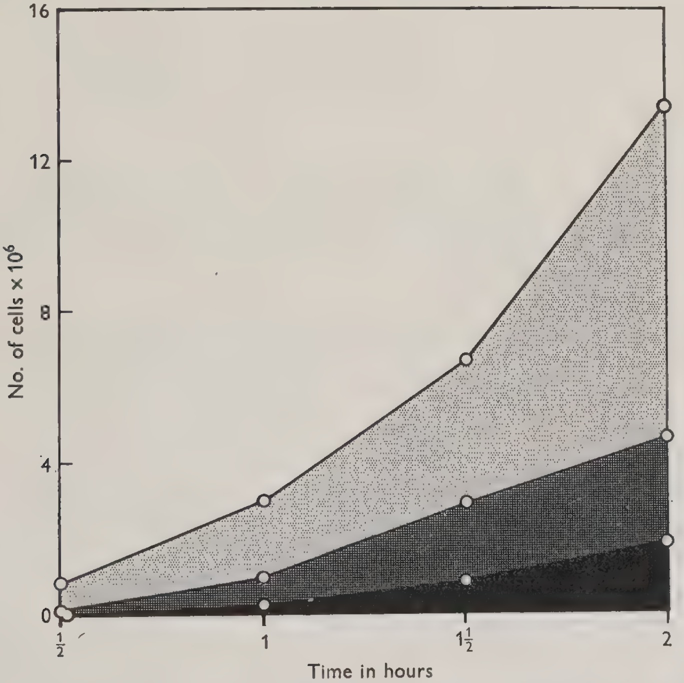

Fig. 10. Cumulative numbers of *Phaeodactylum* cells filtered, ingested and digested over a period of 2 h in the experiments referred to on p. 252. Black area represents digested cells, dark stipple the intact cells within the stomach, light stipple the cells rejected as pseudofaeces, and the unshaded area cells remaining unfiltered.

In (*a*) (Table IX) proportions of the two organisms in the supernatant were estimated before and after the animal had filtered, and, as might have been expected, no significant differences were found, i.e. no selection takes place between organisms as they enter the inhalant siphon. If any noxious or toxic substance is present the animal evidently ceases filtering and closes the shell (see p. 247, *Gymnodinium veneficum*). In (*b*) (Table X) estimates of the proportions of the two organisms were made in the original mixture and in the resuspended pseudofaeces after 6 h feeding. This would give a measure of the sorting by the gills and labial palps, after filtration, but before entry into the mouth. There appears to be a significantly greater proportion of *Peridinium* in the pseudofaeces, and therefore some selection of *Dicrateria* in the food taken in. From experimental results below (Tables XII and XV) we know that *Peridinium* is indigestible, and, further, is much larger (25 μ) than *Dicrateria* (5 μ). There is thus the interesting possibility that sorting by size and weight may take place; it would be desirable, however, to do further experiments before concluding that this type of sorting between different organisms plays an important part in the economy of *Lasaea*.[1] In (*c*) (Table XI) (*Phaeodactylum* / *Chromulina*) estimates were made as in (*b*), after 10 h filtering, but no apparent sorting took place. This would agree with the acceptability of both organisms presented singly and with the absence of an easily sortable organism of very large size such as *Peridinium* in (*b*).

In (*d*) (Table XII) direct estimates were made from the stomach contents of the *Lasaea* after 2 h feeding. Here selection at first sight appears to be in favour of *Thalassiosira*, but when allowance is made for the total disappearance of some *Phaeodactylum* by digestion, the ratio may not be significantly different from that in the original culture.

In the final experiment (*e*) *Lasaea* was fed with a mixture of *Phaeodactylum* and large empty diatom frustules of 'Kieselguhr' (diatomaceous earth). Quantitative determinations were not made, but animals were fixed and sectioned after 2 h feeding, when the distribution of 'Kieselguhr' could be followed carefully. In some animals, though not all, the stomach contained particles of 'Kieselguhr' reaching 30–35 μ in size. By far the greater part of the ingested material was a mass of pure *Phaeodactylum*, while the rejecting areas of the gill margin, labial palps, sides of the foot and ventral edges of the mantle were heavily loaded with diatom fragments. It is clear that, in a heavy concentration of *Phaeodactylum* and large diatom fragments, sorting, though not fully effective, does occur.

Further experimental animals were fed on a mixture of *Phaeodactylum* and 'Aquadag'. Although the present writers would agree with MacGinitie (1941) that this substance produces unnatural feeding behaviour, there is evidently no barrier to its ingestion. Large masses of 'Aquadag' were found embedded in mucus in the stomachs of sectioned animals. The 'Aquadag' did not appear to disperse within the stomach, but to pass in a compact bolus directly to the intestine.

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[1] The *Peridinium*/*Dicrateria* mixture was presented in 'thin' culture so that the fraction taken into the gut and that rejected in the pseudofaeces might be of comparable order of size. Thus any difference in the proportions of the two organisms ingested are unlikely to be obscured by a markedly greater bulk of pseudofaeces.

TABLE IX. PROPORTIONS OF *PHAEODACTYLUM TRICORNUTUM* AND *DICRATERIA INORNATA* REMAINING IN THE SUPERNATANT AFTER 6 H FILTERING

		Ratio		
		Phaeodactylum	:	*Dicrateria*
Control at 10.15 h		1·59		1
Control at 16.15 h		1·67		1
After filtering till 16.15 h	(1)	1·65		1
	(2)	1·53		1
	(3)	1·76		1
	(4)	1·53		1
	(5)	1·46		1
	(6)	1·55		1
Average value for six experiments		1·58		1

TABLE X. PROPORTIONS OF *PERIDINIUM TROCHOIDEUM* AND *DICRATERIA INORNATA* IN THE RESUSPENDED PSEUDOFAECES AFTER 6 H FILTERING

		Ratios		
		Dicrateria	:	*Peridinium*
Control before experiment		2·55		1
In pseudofaeces after 6 h	(1)	2·36		1
	(2)	1·88		1
	(3)	1·53		1
	(4)	1·43		1
	(5)	1·21		1
	(6)	1·51		1
Average value for six experiments		1·65		1

TABLE XI. PROPORTIONS OF *PHAEODACTYLUM TRICORNUTUM* AND *CHROMULINA PUSILLA*, IN THE RESUSPENDED PSEUDOFAECES AFTER 10 H FILTERING

		Ratios		
		Phaeodactylum	:	*Chromulina*
Control before experiment		1·7		1
In pseudofaeces after 10 h feeding	(1)	2·1		1
	(2)	1·98		1
	(3)	1·49		1
	(4)	2·3		1
	(5)	1·92		1
	(6)	1·47		1
Average value for six experiments		1·88		1

Table XII. Proportions of *Phaeodactylum tricornutum* and *Thalassiosira fluviatilis* in the Stomach Contents of *Lasaea rubra* After 2 h Feeding

		Ratios	
		Phaeodactylum :	*Thalassiosira*
Control count of mixture before feeding		7·6	1
Ratio in stomach contents after 2 h feeding	(1)	5·5	1
	(2)	7·5	1
	(3)	6·5	1
	(4)	4·6	1
	(5)	7·5	1
Average value for five experiments		6·3	1

Digestive Activity

Estimation of the digestive powers of *Lasaea rubra* was approached in two ways. First, the enzymic activity of a centrifuged homogenate of the whole animals was tested, using methods cited in Stephenson (1939). From much previous work on the digestive powers of lamellibranchs (see Yonge, 1926; Fox & Marks, in Fox, 1936) it was thought safe to assume the presence of a protease, amylase and lipase among the enzymes of the digestive gland. This was verified by confirmatory spotting tests, and in addition a slowly acting pectinase was found (a wedge of carrot flesh was reduced to pulp after 7 days, control wedges remaining intact). Protein, in the form of dogfish blood corpuscles, was found after 2 h to have undergone extracellular digestion within the lumen of the stomach, as seen in stained sections of *Lasaea*. After 12 h no result was obtained with the absorptiometric determination of cellulase activity (see Stephenson, p. 316) as used by Newell (1953) with *Ostrea*.

Secondly, the stomach contents of *Lasaea* were examined at intervals after feeding with possible natural foods. Experiments of the classical kind, using artificial 'type' substrates, such as cotton-wool, filter-paper and fibrin, in incompletely controlled conditions *in vitro*, seemed less likely to give an exact picture of the animal's natural digestion. The condition of the gut contents at stated times after feeding with the flagellates *Isochrysis galbana* and *Peridinium trochoideum*, and with *Phaeodactylum tricornutum* and *Thalassiosira fluviatilis* is shown in Tables XIII–XV. These results show a rapid and complete digestion of *Isochrysis* and *Phaeodactylum*. Whatever the true structure of the wall of *Phaeodactylum* (see Wilson, 1946, and Hendey, 1954) it is evidently very easily attacked by digestion to release the protoplasmic contents of the cell. The majority of ingested *Phaeodactylum* had become 'ghosts' in 45 min (Table XIV). Easy digestion of these two organisms is accompanied by rapid filtration, and they seem likely to be a nearly ideal food.

By contrast with these easily digested organisms we have the results using a heavily walled diatom, *Thalassiosira*, and an armoured dinoflagellate, *Peridinium*. *Peridinium* is not digested, nor is this surprising, as its digestion would require a cellulase, which *Lasaea* lacks. Further, there is a correlation between low filtering rate and indigestibility. With *Thalassiosira* it was found difficult to make reliable absorptiometric measurements of uptake by *Lasaea*, as its normal habit in thick culture is to lie in a sludge close to the bottom of the vessel, though it is easily stirred into suspension and can thus become available for short periods for filtering. It is found that *Lasaea* can slowly digest *Thalassiosira*, presumably with the aid of the pectinase in the digestive gland, which would aid in separation of the siliceous valves. The process is, however, very slow, and it seems likely that diatoms are not in nature a good or sufficient food.

TABLE XIII. CONDITION OF THE GUT CONTENTS OF *LASAEA* FED WITH VARIOUS ORGANISMS

(Examined in sectioned and stained material.)

Specimen no.	Feeding	Gut contents
1	1 h in *Peridinium trochoideum*	*Stomach*—cell walls of *Peridinium* all intact, many organisms still containing organized stainable protoplasm, somewhat shrunk due to fixation. No digestion. *Intestine*—well recognizable cell walls with contents. No digestion
2	1 h in *P. trochoideum*	*Intestine*—little evidence of digestion. Cell-wall plates still clearly recognizable
3	2 h in *Phaeodactylum tricornutum**	*Stomach*—filled entirely with 'ghost' cells. In a few cells a bluish staining material (azan), probably leucosin rather than protoplasm, remains (see Hendey, 1954). Digestion complete. *Digestive gland lumen*—entirely filled with 'ghost' cells in same condition as those in stomach. *Intestine*—'ghost' cells even more eroded than in stomach. Digestion complete
4	2 h in *Isochrysis galbana*	*Stomach*—no trace of organized protoplasm or intact cells. Digestion complete
5	2 h in *I. galbana*	*Stomach*—digestion complete as in (4)
6	2 h in *Thalassiosira fluviatilis*	*Stomach and intestine*—only a few cells digested, majority intact
7	2 h in *T. fluviatilis*	*Stomach and intestine*—little digestion. Some cells appear empty, but there is little breakdown of cell contents, even in faeces. Cell walls are always clearly defined. Digestion is apparently very slow, and the majority of cells are passed in faeces before digestion
8	2 h in *T. fluviatilis*	Less digestion than in (7). Almost all the cells show clearly defined protoplasmic contents
9	2 h in *T. fluviatilis*	*Stomach*—contained relatively few diatoms, showing only slight traces of digestion
10	2 h in *T. fluviatilis*	*Stomach*—diatoms almost all undigested. *Intestine*—faeces formed of compacted masses of cells, mostly quite undigested

* See also Table VIII, p. 254, and Table XIV, p. 260.

TABLE XIV. CONDITION OF THE STOMACH AND INTESTINE CONTENTS OF *LASAEA RUBRA* AFTER FEEDING WITH *PHAEODACTYLUM TRICORNUTUM*

(Animals fed on *Phaeodactylum* for 3 h, then placed in filtered sea water. Stomachs were dissected from living animals, contents diluted to 0·2 ml. with sea water and counts made.)

Animal no.	Time after feeding			Counts of cells in 0·1 mm^3, after dilution				Total in stomach in thousands	Remarks
1	45 min	Stomach	Whole cells	48	56	28	32	82	Most of the 'ghost' cells heavily eroded, visible only with difficulty
			'ghosts'	64	76	80	64	142	
		Faeces	Whole cells	24	44	56	76	—	Also much detritus remaining from before feeding with *Phaeodactylum*
			'ghosts'	28	52	56	76		
2	1 h 20 min	Stomach	Whole cells	16	8			24	—
			'ghosts'	30	20			50	
		Faeces	Whole cells	0				—	Faeces with large no. of flagellates and detritus, in addition to *Phaeodactylum*
			'ghosts'	4					
3	1 h 45 min	Stomach	All digested to an advanced stage	—				32*	No cells remaining with contents and 'ghosts' much further digested than in 1 and 2—only visible with difficulty
4	2 h	Stomach	Whole cells	2	4			41*	—
			'ghosts'	18	17				
5	2 h 15 min	Stomach	Whole cells	6	7			75*	—
			'ghosts'	28	34				
6	2 h 30 min	Stomach	Whole cells	5	6			51*	'Ghost' cells as fully digested as possible while still remaining visible
			'ghosts'	22	18				

* 'Ghost' and whole cells not estimated separately in total in stomach.

DISCUSSION

Filtering Rates

Although all of the animals, so far as could be detected, kept their valves open and siphons out for most of the experimental period, an important cause of the variations in filtering rate recorded must have been the temporary cessation of activity of some of the animals. An attempt has been made to reduce these variations as far as possible by using 20 animals in each experiment and performing as many experiments as was practicable. It is also thought probable that the highest filtering rates have the greatest significance, and approximate most closely to a natural filtering rate in the field. On this basis, the filtering rate of *Lasaea* in *Phaeodactylum* compares closely with that of an adult (74 mm) *Mytilus californianus*, as estimated by the indirect method of Fox *et al.* (1937) (see Table II). A similar order of values was reported by Willemsen (1952) in adult *Cardium edule* and *Mytilus edulis* by a method very closely comparable to the one used here. Smaller specimens of *Mytilus edulis* have shown a faster

TABLE XV. CONDITION OF THE STOMACH CONTENTS OF *LASAEA RUBRA*, AFTER FEEDING WITH *PERIDINIUM TROCHOIDEUM* AND *THALASSIOSIRA FLUVIATILIS*

(Stomachs were dissected from living animals, contents diluted to 0·2 ml. with seawater, and counts made.)

1. *Peridinium trochoideum*

Animal no.	Fed (h)	Cells	Counts of cells in 1 mm³ after dilution				Estimated total cells in stomach
1	1	Cells with walls and contents intact	9	6*	2	4	1050
		Cells empty	–	–	–	–	0
2	1	Intact	5	1	4	1	550
		Empty	–	1	1	–	100
3	1	Intact	1	1	–	–	*c.* 100?
		Empty	–	–	–	–	0
4	1	Intact	4	1	1	–	300
		Empty	–	–	–	–	0
5	2	Intact	7*	4*	4	2	850
		Empty	–	–	–	–	0
6	3½	Intact	4	1	1	–	300
		Empty	–	–	–	–	0
7	3½	Intact	5	6	3	4	900
		Empty	–	–	–	–	0

* Some cells showing signs of rupture of the wall.

2. *Thalassiosira fluviatilis*

Animal no.	Fed (h)	Cells	Count of cells in 1 mm³ after dilution				Estimated total cells in stomach
1	1	Frustules with contents intact	2	2	–	1	250
		Frustules empty*	1	–	–	–	50
2	1	Intact	1	1	1	–	150
		Empty	–	–	1	–	50
3	2	Intact	1	–	–	–	Very few taken into stomach
		Empty	–	–	–	–	
4	2	Intact	2	1	–	1	200
		Empty	–	1	–	1	100
5	2½	Intact	4	2	–	–	300
		Empty	–	2	–	–	100
6	2½	Intact	2	2	–	3	350
		Empty	2	1	–	3	300
7	4½	Intact	4	3	2	1	500
		Empty	–	2	1	1	200
8	4½	Intact	4	3	3	2	600
		Empty	5	4	1	1	550
9	4½	Intact	7	3	6	6	1100
		Empty	2	3	1	2	400
10	16	Intact	1	–	1	1	150
		Empty	1	–	–	–	50
11	16	Intact	–	1	1	1	150
		Empty	1	–	–	–	50
12	16	Intact	6	3	1	–	500
		Empty	1	1	1	–	150
13	16	Intact	1	1	–	1	150
		Empty	–	2	1	–	150

* Sample counts of original thick culture for proportion of empty frustules: intact frustules 1:5.

rate, Jørgensen's figures giving ratios of *c.* 200 and 150 with animals 15 and 30 mm long respectively. Jørgensen appears to have calculated rates over relatively short periods of up to 1 h. All the *Lasaea* calculations are, however, for rates uniformly sustained for at least 3 h. The high initial rate almost always obtained by Jørgensen with single *Mytilus* was never observed in *Lasaea*. Much higher values were also twice reported with oysters, when the direct method of measuring water pumped out of the exhalant aperture of the pallial cavity was used. Here, if the volume of the animals has been estimated correctly in Table II, the ratio is considerably higher than with the indirect method of measuring filtration.

Control experiments were carried out to determine whether variations in the density of the culture had any effect on the filtering rate. Loosanoff & Engle (1947) suggest that in conditions of high turbidity, feeding, as distinct from filtering, might cease in oysters. Variation in concentration of *Phaeodactylum* produced no significant difference in filtering rates, though in dense cultures the output of pseudofaeces is markedly increased. In the experiments with various flagellates recorded above, no correlation could be found between initial density of the experimental culture and the rate of filtering. It seems likely that a high filtering rate can be maintained at the highest densities used in these experiments, as will be seen from the values with *Chromulina* and *Isochrysis*, which were both used in thick cultures. On the other hand, a thin *Prorocentrum* culture was filtered almost as rapidly as the dense *Isochrysis*. Thus, with pure suspensions of food organisms, the density of the suspension appears to be without effect on the filtering rate.

Of the variations in filtering rate recorded with different organisms, perhaps the most striking result was obtained with the naked dinoflagellate *Gymnodinium veneficum*, in which no sustained filtering was observed at all. The remaining organisms may be arranged—by comparison with *Phaeodactylum*—in two groups. First, *Nannochloris atomus*, *Chlorella stigmatophora*, *Peridinium trochoideum* and *Dicrateria inornata* were filtered at rates significantly lower than that for *Phaeodactylum*. With each of the remaining flagellates, namely *Isochrysis galbana*, *Exuviaella baltica*, *Chromulina pusilla* and *Prorocentrum micans*, the average rate was faster than that for *Phaeodactylum*. The most rapidly filtered organism in any of the experiments was *Isochrysis galbana*, the fastest rate with this flagellate being 2·181 ml./h for 20 animals. The fastest rates with *Chromulina pusilla*, *Exuviaella baltica* and *Prorocentrum micans* were respectively 2·12, 1·184 and 1·365.

It will have been noted that micro-organisms were always presented to the animals at concentrations greatly exceeding those ever present in the sea; and the criticism might be made that the experimental results establish nothing more than the reaction of *Lasaea* to a series of unialgal cultures at artificially high concentrations. There are, however, several indications that the preferences disclosed may have some reality under natural conditions. Thus, with the toxic *Gymnodinium veneficum* no filtering was obtained, and with *G. vitiligo* and *Chlorella stigmatophora*, both suspected by Bainbridge (1953) of mild toxicity, low filtering rates were recorded. However, *Dicrateria* and the minute *Nannochloris*, which also gave low rates, have never been suspected of toxicity. A recent report by Pinto (1953) states that *Exuviaella baltica* and *Prorocentrum micans* have both been found toxic in 'red water', but in our experiments using the Plymouth strains of these organisms we have found no evidence of toxicity. The filtering rates with these organisms were among the highest obtained.

Of the other flagellates filtered rapidly by *Lasaea rubra*, *Isochrysis galbana* has repeatedly been found highly acceptable as food to cultures of filter-feeding animals. *Chromulina pusilla*, a species also filtered rapidly, is probably identical with the 1–2 μ component of the phytoplankton that evidently contributes heavily to the natural food of *Lasaea* (Table XVI). With *Peridinium trochoideum*, on the other hand, there appears to be a correlation between low filtering rate and lack of digestibility (see Table XV).

Any differences in pumping-rate resulting from the different sizes of the organisms, and consequent higher efficiency of filtering large organisms, appear to be unlikely. Small *Chromulina* (1–2 μ) were in fact filtered rapidly, large *Peridinium* (25 μ) slowly. Nor could any correlation be found by plotting the sizes of each of the various species of flagellates against the respective filtering rates obtained.

No figures appear to exist for exact comparison of the filtering rates of other lamellibranchs with different types of flagellates. Jørgensen (1949) carried out experiments with *Mytilus*, using, in addition to *Phaeodactylum*, the flagellates *Dicrateria inornata* and *Isochrysis galbana*. He used single specimens of *Mytilus* of lengths 1·5, 2·9 and 3·2 mm, but his experiments, being not primarily directed towards this point, appear to be too few in number, and the results to fluctuate over too wide a range, to allow significant correlation of feeding rate with type of organism.

Further experiments might well be carried out on filtration rates with various organisms, and on possible mechanisms of discrimination. Bainbridge (1953) studied the effects of various phytoplanktonic organisms on planktonic animals, but little seems to have been done on the relation of sedentary benthic animals to particular constituents of the phytoplankton. With *Lasaea* itself nothing is yet known of the way in which the presence of a particular kind of flagellate exerts its effect on filtering rate. There may be different effects on the rate of beat of the gill cilia, different degrees of contraction of the gill mesh, or variations in the proportion of time engaged in filtering. It is hoped to carry out further experiments on some of these points with the use of a larger bivalve such as *Mytilus*. Of particular interest as a first study would be the direct observation of the action of various phytoplankton cultures on ciliary beat.

The Filtering Efficiency of the Gill

Jørgensen (1949) and Jørgensen & Goldberg (1953) discuss the mechanism of collection and transport of food particles by the lamellibranch gill, and whether this takes place by a continuous mucous sheet moving over the gill surface, as suggested by MacGinitie (1941), or with the aid of only a small amount of mucus. Using *Mytilus edulis*, Jørgensen (1949) found that, under certain conditions, graphite particles were filtered down to 1–2 μ in size, and at other times the majority of the $< 5\,\mu$ particles were rejected. He explains these differences by the presence or absence of a 'mucous net' constituting a fine filter upon the surface of the gill. When feeding takes place by this net, particles become intercepted by the mucus and little or no selection according to particle size is possible. Under certain conditions, however, the smaller sizes of graphite particles are allowed to pass through the gill ostia in the absence of a mucous net, at which times filtration will take place merely by the gill ostia or the efficiency of the straining cilia. Later Jørgensen & Goldberg (1953) found retention in *Ciona* and in *Mytilus* of graphite particles of 1–2 μ. In *Crassostrea virginica*, 2–3 μ particles were retained, while 1–2 μ particles appeared to pass through the gill. It was assumed that in *Ciona* and *Mytilus* a mucous net contributed to food collecting, after the manner described by MacGinitie, and that in *Crassostrea* the filtering efficiency of the gill was decreased in the absence of mucus.

Our experiments with *Chromulina pusilla* (1–2 μ diameter) and with bacteria showed no evidence of a falling off in efficiency of filtering in *Lasaea* with smaller particle size. With one exception, the 5 μ flagellate *Isochrysis galbana*, the rates with *Chromulina* were the highest recorded. The great potential value of these small and unarmoured forms seem to necessitate a high retentive efficiency of the gill, since it is likely that *Lasaea* feeding in nature must derive a high proportion of its food from abundant flagellates of this size (see Table XVI).

Jørgensen (1949) found evidence that *Mytilus* at times rejected 'Aquadag'. In general *Lasaea rubra* took up 'Aquadag' very vigorously over experimental periods of 5 h, sometimes at a rate as fast as any recorded with flagellates. Freshly made up 'Aquadag' gave contradictory results. In one set of experiments, the same effect was obtained as by Jørgensen & Goldberg (1953); 'new' 'Aquadag', made up for not more than 1 h was poorly filtered, while 'old' 'Aquadag' that had been left to stand for 7 h before use was filtered more than twice as fast. Jørgensen and Goldberg ascribed this effect to the tendency of particles to form larger aggregates on standing. In *Lasaea* the efficient filtering of the smallest particles of 'Aquadag' is probably not conclusive evidence of the presence of a mucous net; and it seems that bivalves may be very differently sensitive at different times to stimulation or irritation by graphite. This irregularity contrasts with the constant and easily reproducible filtering rates obtained in *Lasaea* with suspension of natural foods.

The mesh size of the gill is by no means easy to determine in fixed and contracted sections. In *Lasaea rubra*, moreover, neither the secretion of a mucous net nor the contraction of the gill ostia may in fact be necessary to retain small flagellates. A far more efficient means of entrapping particles at the frontal surface seems to be the fine fringes of eu-latero-frontal and pro-latero-frontal cilia, which are very highly developed in *Lasaea* (see the papers of Atkins, 1936, 1938, for lamellibranchs in general; and Oldfield's recent work, 1955, on *Lasaea rubra*). In previous discussions about the mucous net, the presence of these filtering cilia has been largely ignored, only Verwey (1952) having called attention to their use. Further, there is the strong objection to the mucous-net theory, that such a continuous sheet covering the gill would impede or prevent the action of the elaborate systems of sorting cilia. Jørgensen (1949) has recognized this difficulty; no one has satisfactorily answered it. However valuable MacGinitie's work in this field may be, his interpretation fails to reconcile the freedom of action of the sorting cilia with the employment of a continuous mucous net. It seems at least that efficient filtering can be carried out in the absence of a mucous net, and that the greatest stimulus to mucus secretion when it occurs may be the presence of large amounts of indigestible debris. Certainly, the lack of obvious mucus in many lamellibranchs, which sort particles on the gills and palps, is very striking in comparison with the large amounts of mucus in ciliary-feeding gastropods (Yonge, 1938), *Amphioxus* and ascidians (Orton, 1914), none of which have highly developed sorting mechanisms on the gill surface.

Tammes & Dral, in a recent paper (1955), make a further criticism of McGinitie's theory of an 'ultra-sieve' and take the view that material may be retained by the gill, by a mechanism of adhesion to sticky cilia.

Comparison of Filtering, Feeding and Digestion

In a lamellibranch living in a high concentration of suspended material, it is important to distinguish three separate activities, filtering, feeding and digestion; and the rate of any of these functions will not necessarily be deducible from that of any other. It may be assumed that the formation of pseudofaeces in thick cultures is a consequence of the mechanical overloading of the gills and palps with a greater accumulation of particles than can be received into the gut (see Popham, 1940, for details of rejectory mechanisms of the mantle cavity). Probably because of the great quantities filtered, the pseudofaeces of *Lasaea* were not compacted in strings as in *Mytilus* and *Ostrea*, but lay in a thick sludge, surrounding each cluster of bivalves, removed a little way from the animals by the force of the exhalant current. True faeces were produced in very much smaller amount than pseudofaeces, and had the form of compact oval pellets bound with mucus. Even in the thickest cultures that could be measured by the absorptiometer, it is unlikely that more than a very small fraction of organisms of *Phaeodactylum* size pass through the gill unfiltered; and in the present calculations this has been neglected and complete filtration assumed.

References in many papers to 'feeding' in lamellibranchs must clearly be interpreted as no more than 'filtering'. 'Food' is that which enters the mouth. Often, however, especially in experiments with 'thin' cultures, and probably as a general rule in natural sea water, feeding may approximate closely to filtering. It is also unsafe to speak of 'feeding' as equivalent to 'digestion'. Sometimes, such as in experiments using cultures, the amount of food digested may—with sufficient elapse of time—correspond to that ingested. But in other conditions, and probably in most situations in nature, much material can be shown to be ingested that will never be digested, either by reason of its own nature, or because of the overgorging of the animal's gut.

In several features *Lasaea rubra* appears to present an exception to the classical picture established by Yonge (1926) and later investigators of lamellibranch digestion. These aspects will be more fully discussed in a forthcoming paper. First, there is an entire lack of amoebocytic action within the stomach at any stage of digestion. Secondly, a large amount of extracellular digestion of phytoplanktonic organisms takes place within the stomach, before the products are absorbed, presumably for completion of digestion, by the digestive gland. In so far as this may involve proteolytic enzymes, it is thought that these must originate from the spheres passed into the stomach by holocrine fragmentation of the digestive cells. Probably in these features, as in much else, *Lasaea* is to be regarded as an exceptional lamellibranch, specialized by its small size and as a result of its 'difficult' habitat. It would certainly be unsafe to draw from it at this stage too many general conclusions about the nature of lamellibranch digestion.

The Animal in Nature: Filtering Rate and Feeding Efficiency

From several considerations it seems justifiable to regard the filtering rates observed with *Phaeodactylum* and some of the flagellates as not falling significantly short of the average natural rate. The animals used displayed normal visible activity and posture, and this was taken to imply normal filtering activity with little disturbance by experimental conditions. The relation of the volume of water filtered per hour to the volume of animal showed impressive agreement with figures recorded for other lamellibranchs (see Table II). Further, the filtering rate was found to vary predictably with temperature, with different kinds of flagellates, and in some cases with position on the shore (see below p. 271). It was at least true that variations due to such factors as these were not masked by depressing factors operating as the result of laboratory conditions.

It was also thought safe to assume that there is continuous filtering during the rather short period (*c*. 3 h) that upper tidal *Lasaea* are normally submerged on the shore. In experiments lasting 8–10 h, little falling off in the rate of activity was observed.

The quantity and composition of material suspended in natural sea water, varies enormously with the locality, the phases of the tide, the season of the year and many other factors. Verwey (1952) provides a good discussion of the causes of such variation. The figures provided in Table XVI were obtained by haemacytometer counts of centrifuged sea water in which *Lasaea rubra* was naturally feeding, on the Hoe Front at Plymouth. Two such counts are given, (*a*) from sea water taken at the surface on an incoming tide, about a foot above the rocky bottom, and (*b*) from sea water taken by pipetting from approximately 1 cm above the surface of the rock. In composition and number of organisms these figures may be regarded as representative of water samples from near the substratum in the late summer. No information is at present available for other times of the year.

TABLE XVI. ESTIMATES OF AMOUNTS OF LIVING ORGANISMS AND SUSPENDED MATTER IN WATER SAMPLES FROM ROCKS BELOW PLYMOUTH HOE

(*a*) From surface sea water, a foot above the substratum (12 August 1954) and (*b*) from *c*. 1 cm above the surface of the substratum (20 September 1954).

	Counts per 1 mm^3 natural sea water					
	(*a*)		(*b*)			
Organisms under 2 μ	60	48	27	38	37	29
Organisms 2–5 μ	1	2	3	4	3	3
Organisms 5–10 μ	–	3	1	–	–	2
Totals	61	53	31	42	40	34
Over 10 μ	Much unidentifiable organic debris, sand grains and detritus		*Skeletonema*; several naviculoid diatoms; 3 chrysophycean flagellates; 1 euglenoid; fragments of *Enteromorpha* and '*Ectocarpus*'; and much unidentifiable debris			

Any assessment of the annual food intake and the growth and metabolic requirements of *Lasaea* must await further work than is recorded here. We would now do no more than point out that, if we assume the late summer figures for density of phytoplankton given in Table XVI to approach a representative average for 12 months, and if we take the rate of filtering derived in this paper as coming close to the natural one in the field, then—working from living flagellates and diatoms alone—we find an annual food intake of no more than *c*. 0·07 mg for an animal weighing 1·0 mg. This figure, which—as was found by Fox & Coe (1943) with similar calculations for *Mytilus*—is obviously inadequate, neglects other important sources of food. There is first the considerable amount of organic detritus, revealed in Table XVI and in analyses of stomach contents, that must be stirred into suspension during feeding, and which it is difficult to estimate even in samples from near the bottom. While it is unlikely that *Lasaea* augments its suspended food by sucking up detritus directly from the bottom (neither the gill nor the inhalant siphon show any of the adaptations characteristic of deposit feeders—see Yonge, 1949), particles of debris must be constantly stirred into temporary suspension, and will appear in large amounts in the food of a suspension feeder. There is further the rich spring outburst of phytoplankton, at which time the greatest growth of *Lasaea* must occur; there is also the enormous crop of gametes liberated by intertidal algae, which may densely cloud the water for short periods. Finally, the effective filtering time must often be extended by the lodgment of splash in crevices containing *Lasaea*. Even two or three drops of splash may be enough to stir up significant quantities of detritus from the substratum and make it available for filtering. The smaller the amount of splash—within reasonable limits—the greater will be the concentration of the suspension filtered.

An analysis of the stomach contents of *Lasaea rubra* feeding on the Hoe Front (see Table XVII) is quite comparable with previous reports of food ingested by lamellibranchs (see, for example, Fox & Coe, 1943; Coe, 1947). There is obvious difficulty in finding traces of minute or naked flagellates even a short time after their reception into the stomach, and the total of these ingested is best assumed to be the total amount present in, and filtered from, natural sea water. Their digestion by the animal is probably rapid and complete (see Table XIII). In addition, the stomach often contains diatoms sometimes of large size, up to 50μ, either intact and little digested, or as open or broken frustules. Sponge spicules and siliceous spines of various kinds are a prominent item. Much organic detritus is also present, amorphous in nature, and almost always unidentifiable. In *Lasaea*, as in other lamellibranchs, it forms a normal part of the material taken into the stomach.

On the digestibility of organic debris it is difficult to assemble exact evidence. We have relied here on a careful examination of stomach contents and faeces in stained serial sections. Massive debris almost always appeared much less plentiful in the faeces than in the stomach contents. Faecal pellets were composed chiefly of fine broken spicules, occasional inorganic fragments such as small sand grains, and large diatom frustules, either empty or with stainable contents. It seems that some at least of the large fragments of organic detritus taken into the stomach are broken down by extracellular enzymes and perhaps assimilated. Some breakdown has probably taken place outside the animal by bacterial or autolytic action, and some of the products of this must be available to the animal. *Lasaea* is probably, however, without power to initiate the breakdown of cellulose walls (see results of the enzyme experiments, and experiments with *Peridinium trochoideum*, p. 259). Of diatoms, the experiments with *Thalassiosira* indicate that digestion is slower and sometimes incomplete; certainly some of these, as well as armoured dinoflagellates, must pass through the gut intact. The question of the availability of detritus to *Lasaea* is an important one, on which it is hoped to make further and more critical experiments.

TABLE XVII. EXAMPLES OF THE GUT CONTENTS OF TYPICAL *LASAEA RUBRA*, COLLECTED FROM ROCKS BELOW PLYMOUTH HOE AFTER SEVERAL HOURS' FEEDING

(Examined in fixed and stained serial sections.)

Specimen	Region of gut	Remarks
1	Stomach	Large numbers of small, unidentifiable organisms, corresponding in size to the 1–2 μ flagellates of sea water. A number of broken spicules as of sponges. A diatom frustule, *c.* 20 μ, with no contents. A number of frustules of small naviculoids. Little trace of detritus, or unselected bottom deposits
	Intestine	A number of larger, inorganic particles, up to 30 μ in size, evidently rejected by the sorting area into the intestine
2	Stomach	A large amount of unorganized and unidentifiable detrital material. Maximum size of particles, *c.* 20–30 μ. A good deal of organic (or colloidal) material, staining pink with azan
	Digestive diverticulum	No large or identifiable particles
	Intestine	Contents similar to stomach, including some large, irregular particles and many small refractile specks
3	Stomach	Many particles of amorphous, azan-staining, (? organic) debris, particle size 30 μ and less. Several frustules of diatoms, empty, 10–15 μ
	Intestine	Diatom frustules, and several opaque, inorganic particles
4	Stomach	Large numbers of undetermined spicules, as of sponges, with traces of broken diatom frustules. Small, highly refractile, inorganic particles. A large *Coscinodiscus* of *c.* 50 μ diameter, with stainable contents, apparently not digested
	Digestive diverticulum	No identifiable particulate contents
	Intestine	Spicules, detrital particles, and fragments of inorganic material up to 15 μ across. A large *Coscinodiscus*, *c.* 50 μ across, with the contents not digested

Ecology: *Intertidal Distribution*

Morton (1954), in a paper on the animals inhabiting intertidal crevices, has briefly discussed the ecology of *Lasaea rubra*. The species extends essentially throughout the intertidal zone, except the uppermost *Littorina neritoides* fringe. Its upper limit at Plymouth is at about the top of the *Chthamalus* barnacle zone, and it ranges downwards to low-water mark, as was pointed out by Glynne-Williams & Hobart (1952). Morton has recorded that at Wembury it may just reach crevices in the zone of black lichen, *Verrucaria maura*, commenting that 'its presence here is a remarkable achievement for a lamellibranch relying on suspension feeding'.

There is in general a good correlation of shell size with length of time of

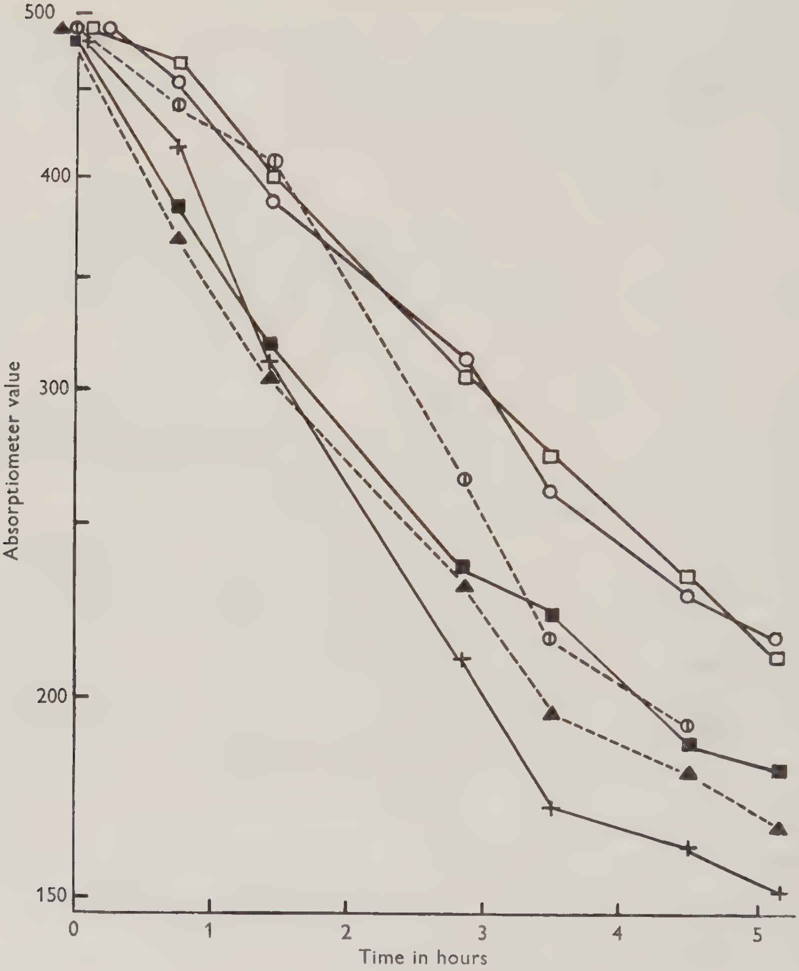

Fig. 11. Filtering rates in *Phaeodactylum* of three series, each of 10 large *Lasaea rubra*, from near low-water mark (location 1), and from the upper limit of the range (location 5–6), at Wembury. □——□, ○——○ and ⦶– –⦶ are from the lower level, and +——+, ■——■ and ▲– –▲ from the upper level. The filtering rate in the high tidal series is initially greater, becoming reduced to that of the upper series after *c.* 3½ h. Of the low-tidal series, ⦶– –⦶ exhibited a higher filtering rate than the remaining two, reaching the same performance as the high tidal series after 1½ h.

submersion. The largest size (3·0 mm) is reached at the lower tidal positions (Morton's locations **1** and **2**); and the smallest adult shells (never more than 2 mm in length) are found in tufts of *Pygmaea*, much higher on the shore (location **8**). The latter habitat seems to be the most harsh which this species has to tolerate. The percentage exposure between tides may exceed 80, and at Wembury, the areas of *Pygmaea* on sun-exposed rock faces become desiccated and brittle between tides during summer. The shells of *Lasaea* become entirely dry. Yet in spite of the apparent difficulties of the habitat, the *Pygmaea* tufts are—as was pointed out by Colman (1940)—the most certain places to collect *Lasaea rubra* in large numbers alive.

In some places there appear to be exceptions to the rule of greater size with longer submersion. One example is shown by the *Lasaea* at Blackstone Rocks, Wembury, at the extreme upper limit of the *Chthamalus* zone, at Morton's locations **5** and **6** (percentage exposure *c.* 90). The shells here are large in modal and maximum size. Their size induced us to do some experiments on their filtering rates as compared with those of animals of comparable size selected from lower tidal levels (percentage exposure 45). The results appeared interesting, in showing a marked initial increase in the filtering rate of those higher on the shore as compared with those lower down (see Fig. 11).

The ability to filter rapidly continued for a longer period than the normal time of submersion or contact with splash, which could never much exceed 2 h. Apparently *Lasaea rubra* living at this high level may be equipped to take rapid advantage by fast filtering of the presence of even the most temporary patches of water lodged by the splash.

The results of these preliminary experiments do no more than to point a field which it is hoped in the future to explore: the study of upper tidal *Lasaea rubra* as a filter-feeding animal living under hard conditions. It would be interesting to find whether *Lasaea* is regularly able to compensate for an unfavourable position on the shore by an increased filtering rate. In future experiments it is hoped to investigate possible differences in growth rate, metabolic rate and filtering rate, correlated with differences in shore level.

SUMMARY

The rates of filtering, feeding and digestion have been experimentally investigated in the small intertidal lamellibranch *Lasaea rubra*. Absorptiometric determinations of filtering rates with cultures of *Phaeodactylum tricornutum* were carried out over 3 h periods. The filtering rate is strongly affected by temperature change, but not—it appears—by variations in the turbidity of the culture. For a group of 20 animals, the filtering rate reached an upper level of more than 1 ml. per hour. A very comparable order of value has been previously established for other lamellibranchs studied.

Variations in filtering rate were investigated with the use of unialgal cultures of other organisms. The toxic *Gymnodinium veneficum* was not filtered at all. *G. vitiligo*, *Chlorella stigmatophora*, *Nannochloris atomus*, *Peridinium trochoideum* and *Dicrateria inornata* were filtered at rates lower than for *Phaeodactylum*; *Exuviaella baltica*, *Prorocentrum micans*, *Isochrysis galbana* and *Chromulina pusilla* were filtered at higher rates. The cell size of the organism—like the density of the culture—had no detectable effect on filtering rate. Samples of *Lasaea* pre-treated with the toxic *Gymnodinium veneficum* showed a subsequent depression of the normal filtering rate with *Phaeodactylum*. Eventual recovery was obtained.

Haemacytometric counts of stomach contents were made after feeding with *Phaeodactylum* and the rate of digestion established over a 2 h period. Preliminary digestion, with the reduction of the cell to a 'ghost', takes place extracellularly.

The ability of *Lasaea* to carry out sorting of mixed suspensions was investigated, after feeding with varied particle sizes, by examination of the remaining supernatant culture, the pseudofaeces and the gut contents. Only with mixtures of *Peridinium* and *Dicrateria* and of *Phaeodactylum* and 'Kieselguhr' does evidence of sorting ability appear.

Lasaea was found to digest *Phaeodactylum* and *Isochrysis* rapidly, the diatom *Thalassiosira* slowly and *Peridinium* not at all. It is thought likely that organic detritus figures largely in its food budget.

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THE EFFECT OF WATER CONSERVATION ON THE STRUCTURE OF MARINE FISH EMBRYOS AND LARVAE

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(Plate I and Text-figs. 1–2)

Fish eggs can be divided into three main groups: marine pelagic eggs which float of their own accord, marine demersal, and freshwater demersal eggs. The embryological development of freshwater teleosts follows an accepted pattern, demonstrated by morphologists during the nineteenth century. Marine embryos, both pelagic and demersal, do not conform in all respects to this pattern, and misunderstandings have frequently arisen by lack of attention to known structural differences between the three groups. McIntosh & Prince (1890), in their comprehensive review of fish development, emphasized some outstanding anomalies in pelagic structure, but gave no adequate explanation of their occurrence.

The present purpose is to draw attention to a striking divergence in the morphology of these three groups; to relate it to the difference in habit; and to demonstrate how it affects the transport of yolk derivatives during the embryonic and larval phases. Here the term 'larva' refers to the yolk-sac stage after hatching. The post-larval phase begins with the final disappearance of the yolk.

The Subdermal Space of Pelagic Embryos and Larvae

Pl. I, fig. 1, is a transverse section through the trunk of a 4-day-old demersal trout (*Salmo trutta*) larva, showing the nerve cord, notochord, urinary vessels, gut and axial blood vessels, all surrounded by the heavy myotome musculature. The integument is closely apposed to the underlying mesoderm, and is extended dorsally and ventrally as the marginal or embryonal fin.

This orthodox picture should be compared with that shown in Pl. I, fig. 2, which is a transverse section through the trunk of a 2-day-old pelagic plaice (*Pleuronectes platessa*) larva. The myotomes are relatively weak, and the integument is well separated from the underlying mesoderm to give a voluminous subdermal space. This space has already been recorded by earlier workers. A day before his cod hatched, Meek (1924) observed: 'During the preceding day or two, a space has been gradually forming on each side of the body, separating the ectoderm from the underlying parts. This space is now a prominent feature of the body and tail. It is the preparation for a subdermal space which forms an important larval feature; it is lined with mesenchyme and is apparently a lymph sinus.' McIntosh & Prince (1890) also described the same space as a late embryonic structure, filled with a jelly-like lymph and extraordinarily enlarged above the cephalic region.

In the cod (*Gadus callarias*) larva (Pl. I, fig. 3), the separation of integument from mesoderm is complete in the gut and pectoral region. There is no separation in front of the eyes and over the mandible, nor posteriorly in the caudal region, where the integument is closely knitted to the lateral mesoderm of the main axis, but separation dorsally and ventrally produces the lumen of the big marginal fin, which must be supported, in view of the lack of fin-rays, by turgor within the space.

When larval fin-rays are absent, or just developing, it is reasonable to suppose that the size of the marginal fin, in relation to the more compact axial structures, will vary according to the extent of the subdermal space. The transverse sections in Pl. I show a marked difference in relative fin size between a pelagic marine larva with a big subdermal space, and a demersal freshwater larva with little integumental separation.

I scoured the literature for drawings of newly hatched fish larvae from the three egg types, to see if there were consistent differences in marginal fin size. Ten representative drawings per group are displayed in Text-fig. 1. The drawings are simplified reproductions from various publications; the outlines are exact. My tentative conclusions from this visual comparison are that larvae from pelagic marine eggs, with consistently big fins, have extensive subdermal spaces, and that these spaces are not well defined in demersal

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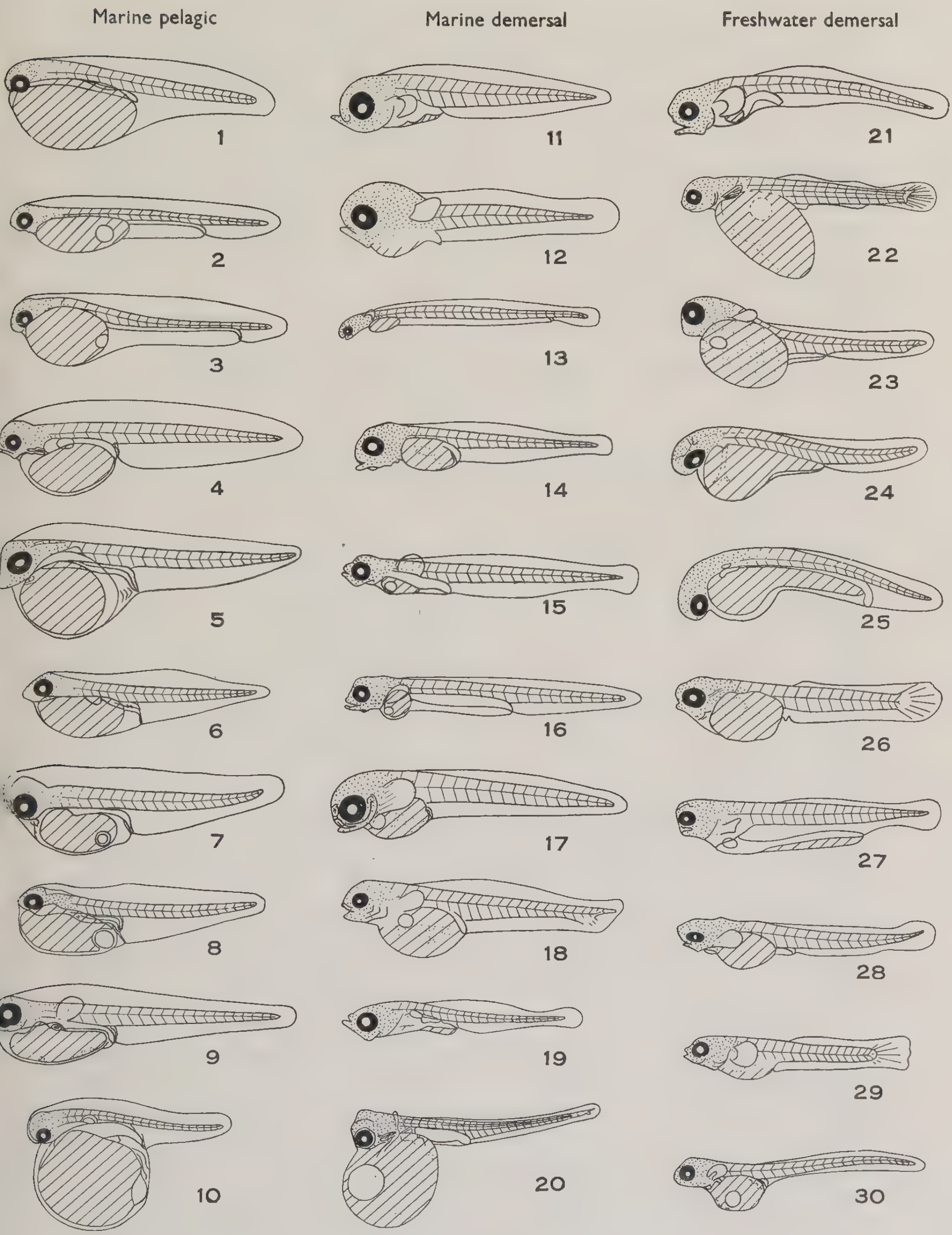
Legend to Text-fig. 1.

Text-fig. 1. A comparison of marginal fin size in early larvae from different types of teleost eggs, i.e. marine pelagic (left), marine demersal (centre), and freshwater demersal (right). 1, *Gadus luscus*, 3·0 mm; 2, *Argentina sphyraena*, 6·9 mm; 3, *Clupea pilchardus*, 3·5 mm; 4, *Pleuronectes platessa*, 8·0 mm; 5, *Solea vulgaris*, 3·2 mm; 6, *Callionymus lyra*, 2·2 mm; 7, *Rhombus laevis*, 4·0 mm; 8, *Scomber scombrus*, 3·5 mm; 9, *Trachinus vipera*, 3·3 mm; 10, *Lophius piscatorius*; 11, *Hypleurochilus geminatus*, 2·1 mm; 12, *Chasmodes bosquianus*, 3·6 mm; 13, *Clupea harengus*, 7·0 mm; 14, *Myoxocephalus scorpius*, 8·2 mm; 15, *Chirolophis galerita*, 12·0 mm; 16, *Pholis gunnellus*, 9·4 mm; 17, *Cyclogaster montagui*, 3·8 mm; 18, *Cottus gobio*, 8·0 mm; 19, *Gobius niger*, 3·0 mm; 20, *Anarrhichas lupus*, 12·0 mm.; 21, *Lota maculosa*, 3·5 mm; 22, *Salmo trutta*; 23, *Gasterosteus aculeatus*, 4·7 mm; 24, *Richardsonius balteatus*, 5·3 mm; 25, *Labeo gonius*, 3·6 mm; 26, *Crenichthys baileyi*, 4·3 mm; 27, *Notropis girardi*, 5·5 mm; 28, *Percopsis omiscomaycus*, 6·0 mm; 29, *Plancterus kansae*, 6·6 mm; 30, *Boleosoma n. nigrum*, 5·0 mm. Nos. 1, 4–10, 13 and 15–20 after Ehrenbaum, 1904, 1905–9; nos. 11 and 12 after Hildebrand & Cable, 1938; nos. 2 and 3 after D'Ancona & Sanzo, 1931; no. 14 after Bigelow & Welsh, 1925; nos. 21, 28 and 30 after Fish, 1931; no. 22 after Stuart, 1953; no. 23 after Ved Vrat, 1949; no. 24 after Weisel & Newman, 1951; no. 25 after Nazir Ahmad, 1944; no. 26 after Kopec, 1949; no. 27 after Moore, 1944; no. 29 after Koster, 1948.


Marine pelagic
Marine demersal
Freshwater demersal

Text-fig. 1.

freshwater larvae, with their consistently small fins. The relative fin size of larvae from demersal marine eggs falls between these two extremes, with *Hypleurochilus* at the pelagic end and *Anarrhichas* approaching the demersal freshwater state. The extent of the subdermal space in this group can be expected to vary accordingly. Larvae from pelagic eggs are usually characterized by integumental separation above the medulla. This feature is the exception rather than the rule in demersal larvae from both environments. Whereas the conspicuous, rayless fin of pelagic larvae persists well into the post-larval phase, the fin of demersal freshwater larvae may begin to assume the adult condition very soon after hatching.

The Subdermal Space and the Problem of Buoyancy in Pelagic Fish Embryos

The development of voluminous subdermal spaces in fish embryos is clearly related to the pelagic habit. The immature egg of the plaice sinks in sea water. Fulton (1898) stated 'that the final changes in the maturation of the pelagic ovum, while still within the ovary, are accompanied by a comparatively rapid and relatively great accession of a watery fluid of low density from the ovary, which dissolves the yolk spherules, is associated with the dissolution of the germinal vesicle and the definite formation of the periblast, distends the ovum to three or four times its former volume, thinning the capsule correspondingly, renders it of crystalline transparency and reduces its specific gravity, so that it is enabled to float in sea water of ordinary density—in other words, to become pelagic....' Milroy (1898) and Dakin (1912) found that the vitelline diluent entering the egg at maturation had a salt concentration with a greater resemblance to adult blood than to sea water.

McIntosh & Prince (1890) described the porous nature of certain fish egg-shells. Krogh, Krogh & Wernstedt (1938) showed that the 'amniotic' or perivitelline fluid of the pipe-fish egg had the same chloride concentration as the sea water outside. The experiments of Jacobsen & Johansen (1908) on the specific gravity of cod and plaice eggs, and the studies of Svetlow (1929) and Gray (1932) on trout eggs, indicate the permeability of the shell to salts and water in these species. If permeable capsules are the general rule among fish, then marine embryos, like their parents, are in constant danger of desiccation under the osmotic gradient. Osmotic work must be done, in the first place, by the peripheral protoplasm surrounding the yolk before gastrulation, and later, by the embryonal integument, in order to prevent loss of water and gain of salts. An unhealthy pelagic egg loses buoyancy in sea water; a dead egg sinks. This rule applies whether the egg has an oil globule or not.

An adult marine teleost replaces water lost under the osmotic gradient by swallowing sea water, absorbing water and some salts through the gut wall, and secreting the excess blood chloride to the outside, through special cells in the gills (Keys, 1931; Keys & Willmer, 1932). This osmo-regulatory mechanism requires an open gut, a well-developed blood circulation and an advanced gill system, for efficient functioning.

The gut of a pelagic plaice embryo opens at a very late stage in incubation (Fullarton, 1891). The blood circulation is ill-developed, as will be shown in the next section, and the gill-bars lack filaments at hatching. The adult osmo-regulatory mechanism cannot apply, in its entirety, to the embryo. A method of direct water acceptance through the integument, to the exclusion of salts, is difficult to conceive in the prevailing osmotic conditions; we must assume, lacking concrete evidence to the contrary, that the permeability of the embryonal integument is normally maintained at a low level, by the expenditure of energy in the form of osmotic work. There is direct evidence to support this assumption in freshwater fish eggs. Krogh & Ussing (1937), using heavy water, confirmed the conclusions of Gray (1932) on the high degree of water impermeability of the trout egg membrane during early development. The evidence for marine eggs is not so substantial. Experiments by Loeb & Wasteneys (1915) led them to believe that the protoplasmic membrane of the fertilized *Fundulus heteroclitus* egg was impermeable to salts and almost impermeable to water. Conversely, Krogh *et al.* (1938) found that unfertilized eggs of *Pleuronectes flesus* and *Crenilabrus exoletus* exchanged water and ions with the surrounding solution. The act of fertilization, however, may have an important bearing on the subsequent permeability of the peripheral protoplasm.

The efficiency of the embryonal integument as a water barrier will depend largely on the health of the embryo and the favourability of its environment. Any osmotic water 'leak' must be followed by adjustment of the internal salt balance. The salt-regulatory mechanism of marine fish embryos and larvae is not yet known, although Krogh *et al.* (1938) showed that such a mechanism must exist in the egg of the pipe-fish *Nerophis ophidium.*

Apart from the question of salt balance, the embryo cannot afford to lose too much water if the egg is to remain buoyant. The limit of water loss, in this context, will be determined by the initial reserve buoyancy at liberation and the loss of weight due to the excretion of respiratory metabolites. A healthy plaice egg remains buoyant until hatching, even though its degree of reserve buoyancy at liberation is not great. If the embryonal weight decreases but little during incubation, then it follows from the simple relationship, mass/volume = density, that little change can take place in the volume enclosed by the embryonal integument. During development, there is a withdrawal of soluble nutrients from the dilute fluid yolk. They are used in the synthesis of the compact embryonic axis, and in respiration. But the low-density yolk diluent remains. It accumulates beneath the integument, forms and fills the voluminous subdermal spaces, and thus maintains an overall embryonal volume in keeping with the buoyancy requirements of the egg.

The problem of water conservation does not arise in a demersal freshwater embryo. One can, therefore, readily understand the lack of a subdermal space in this group, and hence, the ill-developed marginal fin.

A floating marine egg accepts sufficient yolk diluent at maturation to meet both osmotic and buoyancy requirements during early development. A non-buoyant marine egg, on the other hand, requires enough yolk diluent to provide against the osmotic hazard only. Accordingly, one might expect the subdermal spaces of this group to be less capacious than in pelagic eggs; to be more developed than in freshwater eggs; and to vary with specific differences in the reserve of yolk diluent below that necessary to float off the egg. The picture of relative fin size from Text-fig. 1 supports this view.

The Evolution of the Pelagic Egg

The palaeontological record suggests that modern marine teleosts are derived from freshwater ancestors (Romer & Grove, 1935; Moy-Thomas, 1939). The comparative anatomy of adult osmo-regulation is in accord with this view (Baldwin, 1937). There is no reason to suppose that the process of marine colonization by freshwater fish has now ended, nor that reversion back to freshwater is impossible (Hoar, Black & Black, 1951).

Complete physiological adaptation to one or other of these habitats implies the development of a mechanism capable of maintaining the internal environment against the external change, in the egg as well as the adult. The spawning migrations of the salmon, shad and eel can be interpreted as evidence that the embryo is unable to develop in the physico-chemical conditions of adult life.

Assuming teleost embryos to have only a limited tolerance to internal salt change, then what are the essential requirements for the adaptation of a freshwater type of egg to a truly marine existence? First of all, a vitelline membrane highly impermeable to salts and water. The evidence for early impermeability in modern teleost eggs had already been given. Secondly, a means of balancing the internal salt concentration should an osmotic water 'leak' occur in less than optimum conditions. A freshwater embryo, without a functional renal system, must then secrete salts from the outside in. The reverse applies in a sea embryo, and although mechanisms of embryonal salt control are not yet known, it is significant that the chloride secretory cells in the adult *Fundulus heteroclitus* can reverse their polarity when the fish is transferred into fresh water (Copeland, 1948). The ability of the cell membrane to work against a chloride gradient is the essential property of the chloride secretory cell (Hoar *et al.*, 1951). Do such cells occur in fish embryos?

When the external osmotic pressure exceeds that inside the embryo, sufficient water must be present, throughout early incubation at least, to supply the needs of the embryo in the event of osmotic loss. This third requirement is specific to a change from fresh water to marine conditions for egg development. An egg is supplied with water, in the form of yolk diluent, at maturation within the ovary. The specific gravity of teleost body fluids is less than that of sea water. The degree of yolk dilution at liberation may be enough to supply embryonal water needs (in co-operation with a salt-regulatory mechanism) and yet not sufficiently advanced to float off the egg. This is the demersal marine condition. An extension of the process of yolk dilution must inevitably produce a buoyant form of egg, when the upthrust exerted on the distended egg by displaced sea water exceeds the gravitational pull.

Thus, by considering the embryonal adaptations necessary for colonization of the sea, it is possible to arrive at an intrinsically buoyant type of egg. The vast majority of pelagic eggs are, in fact, marine. Buoyancy in the freshwater environment involves a completely different mechanism. This attempt to trace the evolution of buoyancy in fish eggs is intended to be provocative rather than informative, particularly as the mechanism of embryonal salt-regulation is not yet understood and measurements of yolk dilution over a whole range of fish species are not yet available.

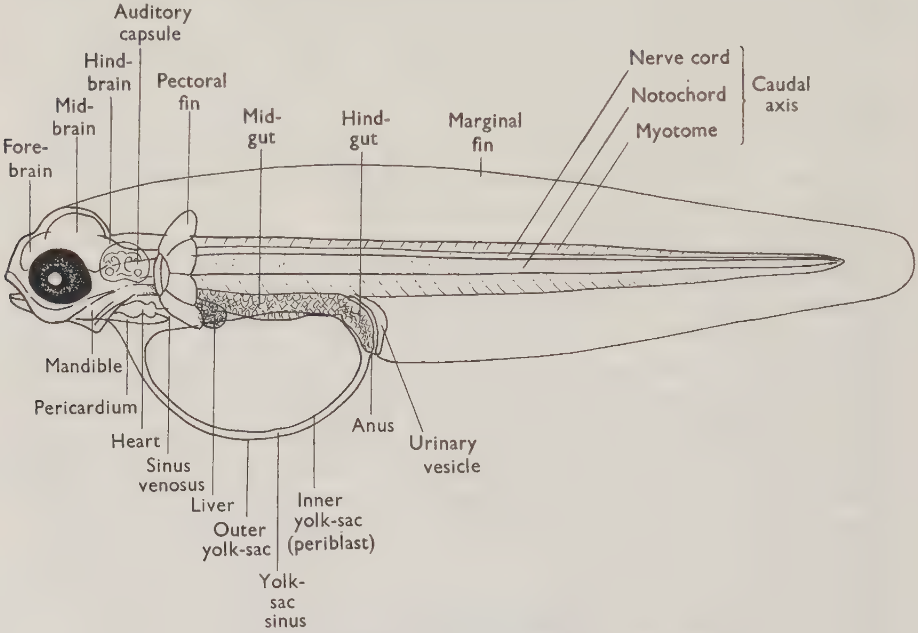
Some marine fish families are more advanced in the direction of pelagic egg production than others. The pleuronectids produce floating eggs only; the gadoids have a demersal representative in *Lota maculosa*. Among salmonids, the trout has a freshwater habit throughout its life-history; the salmon is not yet embryologically adapted to a marine existence; whilst the related *Argentina sphyraena* is completely marine, and has a pelagic egg. There are both demersal and pelagic egg producers among the clupeoids. Early larval representatives of all these families are displayed in Text-fig. 1. Those issuing from pelagic eggs have large, inflated embryonal fins, a feature clearly related to the pelagic habit rather than to family affinities.

The Role of the Subdermal Space in the Transport of Yolk Derivatives

From the time when segmentation starts after the fertilization of a fish egg, to a stage preceding the formation of a pulsating heart and a primitive blood circulation, it is clear that the embryo appropriates yolk by a direct process of incorporation, without the aid of a blood-vascular system. With the subsequent thickening and lengthening of the main embryonic trunk, an increasing volume of tissue is moving out of contact with the yolk in its inner sac, thereby creating problems of food transport.

At this point, the yolk-sacs of most demersal freshwater eggs diverge structurally from those of pelagic marine forms. The former develop a complex system of circulatory vessels around the yolk; in pelagic eggs, this system is always absent, and its function is taken over by the yolk-sac sinus, into which the heart opens directly (Text-fig. 2). The yolk-sac sinus is, in reality, a subdermal space, formed when the extra-embryonal mesoderm around the yolk breaks down into isolated mesenchyme cells during gastrulation (Meek, 1913).

At some stage in embryonal development the ventral yolk-sac sinus is put in lateral communication with the lumen of the rapidly growing marginal fin, on the dorsal side. Pl. I, fig. 3, is a slightly oblique transverse section of a cod larva through the pectoral fin region, showing the gross separation of the integument from the main larval axis, to give a lateral channel connecting the yolk-sac sinus ventrally, to the fin space dorsally.


Auditory capsule
Hind-brain
Mid-brain
Fore-brain
Pectoral fin
Mid-gut
Hind-gut
Marginal fin
Nerve cord
Notochord
Myotome
Caudal axis
Mandible
Pericardium
Heart
Sinus venosus
Liver
Outer yolk-sac
Yolk-sac sinus
Inner yolk-sac (periblast)
Anus
Urinary vesicle

Text-fig. 2 Diagram of a newly hatched plaice larva.

It would seem, therefore, that nutrient derivatives from the yolk of pelagic eggs have easy access to embryonic and larval tissues via the subdermal spaces. To test this supposition I immersed newly hatched plaice larvae in fast fixatives containing corrosive sublimate. A dense brown coagulation quickly appeared in the yolk, the yolk-sac sinus, beneath the integument around the base of the pectoral fins, and in the lumen of the marginal fin. In some normal early larvae, coagulation extended throughout the lumina of both dorsal and ventral portions of the fin (Pl. I, figs. 4, 5). The coagulated moiety of the plasma was frequently washed out of transverse sections during staining. If remaining, it appeared as a decolorized reticulation in the subdermal spaces of the marginal fin, in the cardial and pericardial cavities, and around the base of the pectoral fin.

In case yolk derivatives in the fin were a fixation artifact caused by a strongly contracting yolk-sac, I narcotized five normal larvae in menthol, and measured the length, depth and width of the inner sac. The larvae were then transferred to a mild fixative, Baker's calcium formaldehyde, for 5 min, and the measurements repeated. Later immersion in Heidenhain's Susa intensified the initial coagulation. Table I shows that the inner yolk-sacs of three of the five specimens contracted a little after fixation, but not enough to account for the volume of coagulated derivatives seen in the fin. There was no noticeable movement of plasma from the yolk-sac sinus to the fin during the experiment. The progress of coagulation in larval spaces was watched under a high-power dissecting microscope, and seen to be always from the outside inwards for the dorsal fin, compatible with the view that soluble proteins were already there, before the addition of preservative.

TABLE I. INNER YOLK-SAC MEASUREMENTS IN MICROMETER UNITS

Larva no.	Narcotized			Fixed			Coagulation in Susa
	Length	Depth	Width	Length	Depth	Width	
1	29	31	32	28	31	32+	Heavy
2	10	24	25	10	24	25	Heavy
3	12	25	26	12	25+	26	Heavy
4	46	27	29	43	27+	29	Heavy
5	11	26	32	10	26	31	Heavy

We think of the heart and developing vascular system as becoming vital to the transport of yolk nutrients at an early stage in fish egg development. This may be substantially true for the demersal freshwater egg, with its yolk capillaries and closed vascular system. But in pelagic marine embryos there is an unusual extension of the primary method of direct yolk appropriation, independent of a blood vascular system, well into the larval phase. This is undoubtedly a consequence of the formation of subdermal spaces, dictated by the requirements of water conservation in the marine environment, and of buoyancy.

In the third group of fish eggs, demersal marine, yolk-sac vascularization is as variable as marginal fin development, and probably for the same reason. McIntosh & Prince (1890) related the presence of a vitelline circulation to the demersal habit, irrespective of environment. This is an over-generalization. The herring egg has a yolk-sac sinus and lacks capillaries; *Anarrhichas*, on the other hand, has a conspicuous yolk circulation.

If there is an early separation of the embryonal integument from the inner yolk-sac endoderm, causing the breakdown of the extra-embryonal mesoderm noticed by Meek (1913), then capillary formation becomes impossible. The degree of separation in this region will depend on the volume of yolk diluent left between the two membranes, on the completion of gastrulation. This, in turn, will depend upon the extent of yolk dilution. I have already suggested that variable fin size in demersal marine eggs may be related to variable conditions of water reserve, or yolk dilution. The similar inconsistency in yolk-sac vascularization is in direct line with this view.

In conclusion, it is necessary to reformulate the ideas put forward in this paper, and state that some major differences in the structure and habit of fish embryos and larvae appear to be closely related to a very simple factor, namely, the concentration of soluble nutrients per unit volume of yolk, at the time of egg liberation.

SUMMARY

Fish eggs can be divided into three groups according to habit: pelagic marine, demersal marine, and demersal freshwater. Embryos emerging from these eggs show group differences in the extent of the subdermal space, as inferred from the relative size of their embryonal or marginal fins. Pelagic marine embryos have large, inflated marginal fins, in contrast with the ill-developed fin of the demersal freshwater embryo. The fins of demersal marine embryos lie between these two extremes.

There is a parallel variation in the degree of vascularization of the yolk-sac: pelagic marine embryos have no vitelline circulation, demersal freshwater forms have good vascularization, whilst that of demersal marine embryos is variable.

These differences in both structure and habit can be related to group differences in the degree of dilution of the yolk by ovarian fluid at maturation, and the subsequent need for marine embryos to conserve water under the osmotic gradient. It is possible to construct an argument to explain the evolution of the pelagic marine egg, by considering the embryonic osmoregulatory adaptations necessary for the complete colonization of the sea by ancestral freshwater fish.

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EXPLANATION OF PLATE I

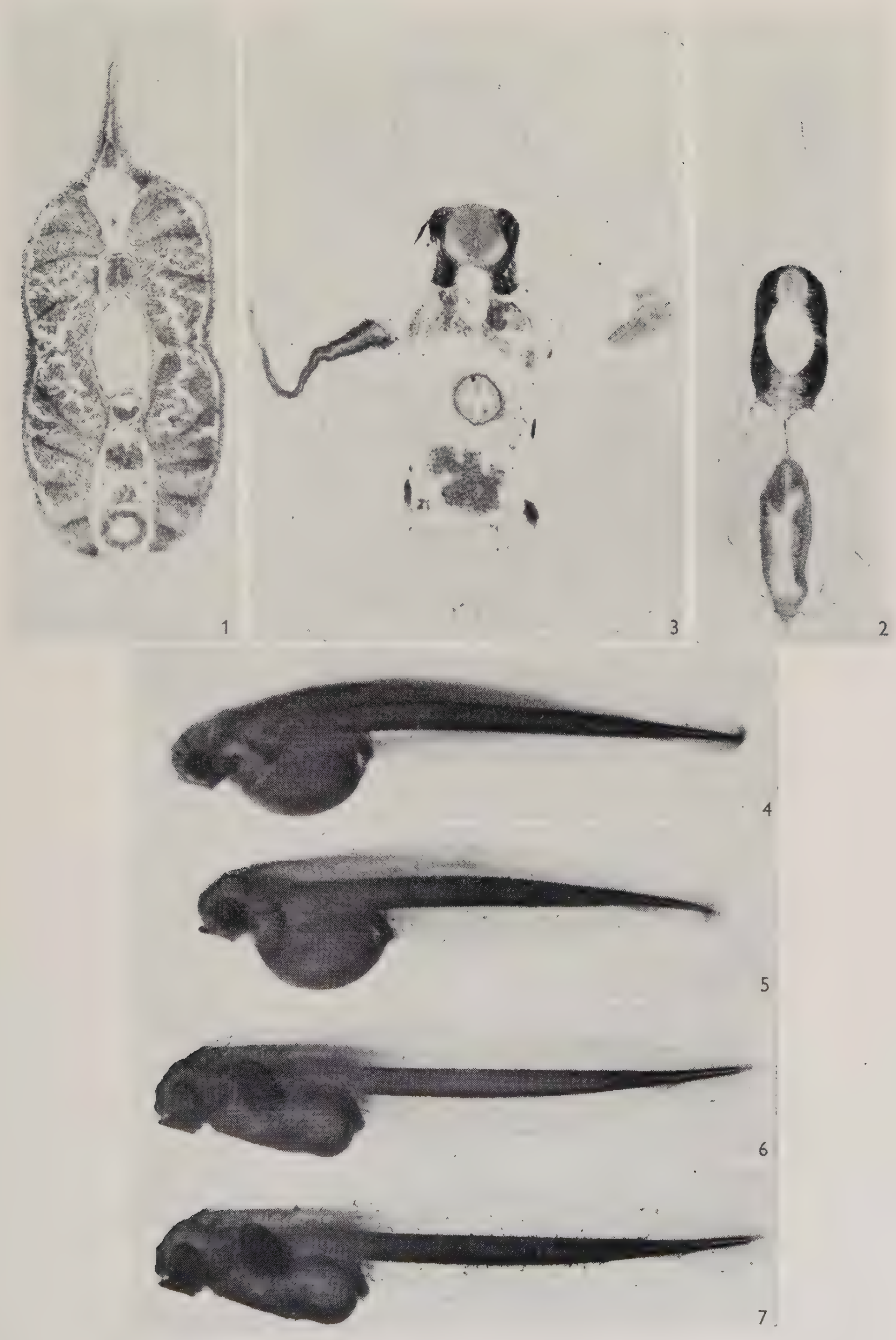
Fig. 1. Transverse section through the trunk of a 4-day-old demersal trout larva, showing the close connexion between the integument and underlying mesoderm. ×50.

Fig. 2. Transverse section through the trunk of a 2-day-old pelagic plaice larva, showing the separation of the integument from the underlying mesoderm to give the superficial subdermal space. ×100.

Fig. 3. A slightly oblique transverse section through the pectoral region of an early cod larva, showing gross integumental separation, and the continuity of the yolk-sac sinus ventrally, with the lumen of the dorsal marginal fin. ×100.

Figs. 4, 5. Newly hatched plaice larvae with extensive coagulation of plasma in dorsal and ventral portions of the marginal fin, after fixation in Heidenhain's Susa. ×12.

Figs. 6, 7. Day-old plaice larvae with heavy plasma coagulation in the anterior part of the dorsal marginal fin, above the head and gut. Fixed in Heidenhain's Susa. ×12.



Figs. 1–7.

ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

Note on the Use of Diphenylbenzidine for the Estimation of Nitrate in Sea Water

By **W. R. G. Atkins**

J. Cons. int. Explor. Mer, 1954, Vol. 20 (2), pp. 153–55

It was shown in 1932 that this reagent, once recrystallized from boiling toluene, can be used for years. The sensitivity is adequate and the colour stable even in bright daylight. All nitrate reagents used in strong sulphuric acid may give low results in presence of organic matter. This error is reduced when 20 mg. of the solid is dissolved in strong sulphuric acid and 7·5 ml. is run into a tube. The sample, 2·5 ml., is then added and is well stirred. The blue colour produced is an oxidation, not a nitration. The reaction is useful also in testing river and well waters and is quicker than the production of picric acid—a nitration product—in the usual test. Methods for the removal of traces of nitric acid from sulphuric acid were cited. Ramachandran heats with a very little formaldehyde, which goes to formic acid, or Pfeilsticker's ammonium chloride method may be used. With known amounts of potassium nitrate a relation may be found between concentration and intensity and this allows for impurity in the sulphuric acid also. W.R.G.A.

Use of the Globe Photometer

By **W. R. G. Atkins** and **H. H. Poole**

Nature, Lond., 1955, Vol. 175, p. 1003

The illumination in a shaded site may be expressed as a percentage of the diffuse light received, from the whole sky, upon a horizontal plate or one may measure the effect at a point, by the action of light on uranium oxalate solution in a flask. The latter is preferable when studying the effect upon plant growth. We had used a selenium rectifier cell under the usual opal diffusing plate, surmounted by an opal hemisphere and an opal globe, so as to render the disc equally sensitive to light at any angle. More recently we assembled a globe photometer with a thin-film caesium-on-silver-oxide vacuum photo cell, a type proved constant in sensitivity for over three years on a roof. This is free from temperature error and always gives a rectilinear relation between current and illumination. W.R.G.A.

Identification of Water-Masses by their Suspended Matter

By **W. R. G. Atkins** and **Pamela G. Jenkins**

Nature, Lond., 1955, Vol. 175, p. 951

For such identifications temperature, salinity and oxygen concentration have been used, also in special cases silica or hydrogen ion concentrations. Jerlov has shown the value of the visual determination of scattering in the Tyndall beam, which Poole and Atkins have measured using a photomultiplier tube. From September 1951 to July 1954 we filtered water from station E 1 through collodion discs. The albedo of the discs gave a good measure of the suspended matter. Apart from the living cells, the basis of the suspension was an amorphous mud, grey, yellowish or chocolate. The albedos were determined with a photo-electric disc, or visual comparisons were made by matching against Klincksieck's or Ridgway's plates. A. G. Lowndes examined the suspended particles using a petrological microscope, and especially important was his finding of microcline on discs from E 1 on 29 March, 1954. It was most abundant on the 5 m disc, which suggests that it had come out in fresh water and was slowly settling. The colour tests suggested the Permian Red Sandstone between Torquay and Exmouth as the source of suspensions found in October (1952 and 1953). W.R.G.A.

Observations on Luminescence in *Renilla* (Pennatulacea)

By **J. A. C. Nicol**

J. exp. Biol., 1955, Vol. 32, pp. 299–300

A study has been made of the luminescent responses of the sea pansy *Renilla köllikeri*. This is a pennatulid found in shallow water along the Californian coast. The responses take the form of light-waves which run over the surface of the rachis. They are controlled by an unpolarized nerve net, and are subject to facilitation.

By photo-electric recording it has been possible to analyse certain details of the luminescent response. At moderate rates of stimulation, several electrical shocks are usually necessary to evoke a luminescent wave, and subsequent flashes increase in intensity (facilitation). By lowering the frequency of stimulation, it is found that more stimuli become necessary to produce the first flash, owing to decay of facilitator.

Maximal estimates of latent period and flash-duration are of the order of 0·5 and 0·9 sec. respectively.

With repeated stimulation the luminescent response of *Renilla* becomes fatigued. Luminescence, furthermore, is inhibited by illumination. After exposure to light the ability to luminesce is lost and slowly recovers over the course of an hour.

Facilitation normally occurs terminally, at the level of the photocytes. By cutting the animal in various ways it has been possible to produce preparations in which neuro-neural facilitation becomes evident. This is occasioned by internuncial fatigue at residual interneural junctions. Normally, however, transmission is 1:1 throughout the nerve net. J.A.C.N.

BOOK REVIEW

THE CHEMISTRY AND FERTILITY OF SEA WATERS

By **H. W. Harvey Sc.D., F.R.S.**

Cambridge University Press 1955

This forms a useful companion to two earlier books by the same author and deals with the physics and chemistry of sea water, especially as these affect or are affected by plant and animal life. The aim throughout is to deal with factors affecting the productivity of the sea and it contains much hitherto unpublished information.

The first part has a short introduction on the composition of sea water and the currents which cause transport and mixing. The changes caused by plants and animals are described, especially the carbon, phosphorus and nitrogen cycles, as well as the role of bacteria in effecting these changes. There is an account of the influence on plant growth of various physical factors. Of special interest is the section dealing with nutrients including those less known such as vitamin B_{12}. The dependence of animal communities on plant production is outlined.

The second part deals with the chemistry of sea water, including both major and minor constituents and the dissolved gases; there is a full account of the carbon dioxide system. A very useful section (in conjunction with F. A. J. Armstrong) gives details of the methods for estimating silicate and the different forms of phosphorus and nitrogen.

There is a good bibliography covering the most important recent literature.

A.P.O.

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J. mar. biol. Ass. U.K. (1956), 35, 291–305
Printed in Great Britain

A CONTRIBUTION TO THE BIOLOGY OF *IANTHINA JANTHINA* (L.)

By Douglas P. Wilson, D.Sc.,
and M. Alison Wilson
The Plymouth Laboratory

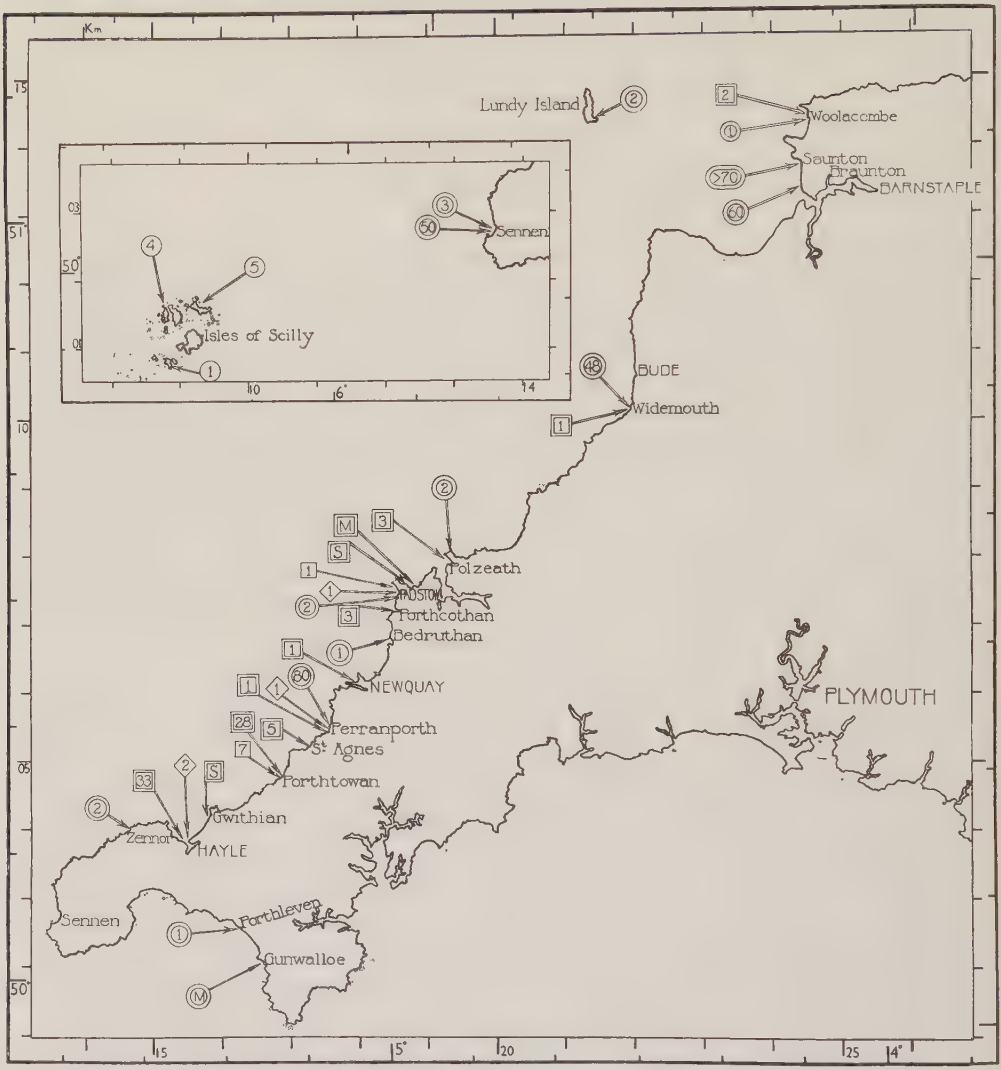
(Plate I and Text-figs. 1 and 2)

The summer of 1954 will long be remembered for lack of sunshine, excess rain and frequent high winds over England and Wales. In the south-west of the region the winds from June onwards until the end of the year were predominantly westerly, often reaching gale force, and the total run of the winds was often above average. During the last week of July there were persistent westerly winds from well out in the Atlantic, often strong and reaching gale force at times, particularly on the 27th and 28th. During the last 4 days the winds were generally from the north-west at Scilly and in southern Ireland. They were variable in strength and direction early during the first week in August, but occasionally blew freshly from the south-west. On the 7th it was often blowing strongly from the west, a gust of 50 knots being recorded at Scilly. It was during this first week in August that the first few specimens of *Ianthina janthina* (Linnaeus) came ashore, heralding the most extensive strandings of this species on British shores for very many years.

It is the purpose of this paper to put on record more data than have been available for any previous stranding in England, and to add some observations on the living animal, whose habits are not yet fully known.

It was in mid-August that we first noted the presence of this oceanic surface-living mollusc in the Padstow district of north Cornwall, where we had been shore-collecting since the early part of the month. Letters published in *The Western Morning News* and *The Times* on 26 August 1954, describing the mollusc and appealing for information of possible strandings in other districts, brought a number of replies, accompanied by actual specimens or by drawings or descriptions which left no doubt of the identity of molluscs seen by untrained observers, who ranged from holiday visitors, old and young, to beach-combers of long experience. A list of the records, with relevant information, is given below, together with our own observations on strandings. For a better appraisal of the extent and timing of the latter they are plotted on the chart reproduced in Text-fig. 1.

To check whether there had been strandings in southern Ireland a letter was sent to *The Irish Times* and published on 4 September 1954. Among replies from people who had found *Ianthina* there before the only one that gave information for 1954 came from Miss Mildred Sheridan, Achill Island, Co. Mayo, who stated that in the previous May, after a particularly violent storm, the strand was covered with the shells. She picked up forty to fifty perfect


Km
15
51°
10
05
50°
15
5°
20
25
4°
Lundy Island
2
2
Woolacombe
1
>70
Saunton
Braunton
BARNSTAPLE
60
03
50'
01
10
6°
14
3
50
Sennen
4
5
Isles of Scilly
1
48
BUDE
Widemouth
1
2
M
3
S
Polzeath
1
1
2
3
PADSTOW
Porthcothan
Bedruthan
1
1
NEWQUAY
1
1
80
Perranporth
28
5
St Agnes
7
Porthtowan
33
2
S
2
Gwithian
Zennor
HAYLE
PLYMOUTH
Sennen
1
Porthleven
Gunwalloe
M

Text-fig. 1. Map to show positions of strandings of *Ianthina janthina* (L.) listed in Table I. Records for the same locality in the same week are summed. Figures indicate the numbers reported, and where actual numbers are not available S = several, and M = many. Numbers or letters within a single circle are strandings during the first week (1–7 August); within a double circle the second week (8–14 August); within a single square the third week (15–21 August); within a double square the fourth week (22–28 August); within a diamond the fifth week (29 August–4 September). Arrows point only approximately to the positions of strandings which are often not known to within 2 or 3 miles.

TABLE I. STRANDINGS OF *IANTHINA JANTHINA* (L.) DURING AUGUST AND SEPTEMBER 1954

Date	Locality	Number	Condition	Recorder
First week (1–7 Aug.)				
Early Aug.	St Agnes, Isles of Scilly	1	Living	Mrs M. Hicks
First week Aug.	St Martins, Isles of Scilly	5	—	Mrs L. M. Hughes
Early Aug.	Tresco, Isles of Scilly	4	Empty shells	Mrs Ariadne Cook
Second week (8–14 Aug.)				
8 Aug.	Gwenver, near Sennen	1	Living	Rev. P. H. T. Hartley
9 Aug.	Treveal Cove, Zennor	2	Living	Miss Tamsin and Master Timothy Procter
9–10 Aug.	Constantine Bay, near Padstow	2	—	Mrs O. Woosnam-Mills
10–11 Aug.	Widemouth Bay, near Bude	48	—	Mrs L. F. J. Gunn
11 Aug.	Saunton Sands, near Barnstaple	*c.* 24 and more on following days	Living	Mrs Doris Wilson
11 Aug.	Gwenver, near Sennen	2	Living	Rev. P. H. T. Hartley
10 or 11 Aug.	Dollar Cove, Gunwalloe	Several dozen	Living	Mr Frank Sabin
About 10–12 Aug.	Sennen Cove	*c.* 50	Living	Mr T. G. W. Fowler
12 Aug.	Braunton Sands, near Barnstaple	*c.* 60	—	Mrs Wiseman
About 12 Aug.	Saunton Sands, near Barnstaple	40–50	Empty shells	Lt.-Col. L. T. G. Ricketts
Second week	Lundy Island	2	One dead	Miss J. L. Bloom
Second week	Woolacombe	1	—	Mr J. Crowder
Estimated second week	Perranporth	*c.* 80	Living	Mr S. Chenoweth
Second week	Coast near Polzeath	2	Living	Mr G. Pym
14 Aug.	Porthleven	1	Living	Mr H. B. Sargent
14 Aug.	Bedruthan Steps, near Padstow	1	Living	Mr T. Roberts
Third week (15–21 Aug.)				
14–21 Aug.	Porthtowan	About 1 a day	Living	Mr N. A. Wesley
15 Aug.	Trevone Bay, near Padstow	3 or 4	Living	Mr C. Roberts
15 Aug.	Trevone Bay, near Padstow	1	Living	Master R. J. H. Wilson
19 Aug.	Trevone Bay, near Padstow	Several	Living	M. A. and D. P. Wilson
19–20 Aug.	Trevone Bay, near Padstow	Several	Living	Miss Bridget Hickey
20 Aug.	Constantine Bay, near Padstow	1	Living	Mrs O. Woosnam-Mills
20 Aug.	Harlyn Bay, near Padstow	Many	Living	Miss H. Bibby
21 Aug.	Harlyn Bay, near Padstow	*c.* 36	Living and recently dead	M. A. and D. P. Wilson
Fourth week (22–28 Aug.)				
22–28 Aug.	Porthtowan	About 4 a day	Living	Mr N. A. Wesley
22 Aug.	Hayle	3	Living	Miss Hartnoll
22 Aug.	Mother Ivey's Bay, near Padstow	2	Living	Mrs O. Woosnam-Mills
22–24 Aug.	St Agnes, near Perranporth	3–5	—	Mr W. E. Williams
23 Aug.	Mother Ivey's Bay, near Padstow	1	Empty shell	Mrs O. Woosnam-Mills
23 Aug.	Daymer Bay, near Padstow	1	Living	Mr S. Lees
23 Aug.	Trevone Bay, near Padstow	Several	Living	M. A. and D. P. Wilson
24 Aug.	Polzeath	2	One living	Mrs E. M. Davies
24 Aug.	Gwithian	1	Living	Dr W. D. Oliver
About 24 Aug.	Widemouth Bay, near Bude	1	Living	Dr E. N. Rudland
24 Aug.	Constantine Bay, near Padstow	1	Empty shell	Mrs O. Woosnam-Mills
24 Aug.	Gwithian	Several	Living	Mrs S. Bennett
25 Aug.	Trevone and Harlyn Bays, near Padstow	Few	Living	M. A. and D. P. Wilson
25 Aug.	Holywell Beach, Newquay	1	Living	Mr P. Rayner-Smith
25 Aug.	Perranporth	1	Living	Miss D. W. Hill
25 Aug.	Porth Kidney Sands, Hayle	*c.* 30	Some living, some empty	Miss Tamsin and Master Timothy Procter
27 Aug.	Gwithian	6	—	Mrs S. Bennett
27 Aug.	Porth Mear, Porthcothan	3	—	Miss H. M. Spittle
27 Aug.	St Agnes, Isles of Scilly	1	Empty shell	Mrs M. Hiels
Before 28 Aug.	Woolacombe	1 or 2	Living	Miss Jane Asher
Fifth week (29 Aug.–4 Sept.)				
29 Aug.	Lelant, near Hayle	2	Empty shells	Mrs D. C. Bazeley
29 Aug.	Constantine Bay, near Padstow	1	Living	Mrs O. Woosnam-Mills
End of Aug.	Perranporth	1	—	Mr S. Chenoweth
End of Aug.	Bryher, Isles of Scilly	1	Empty shell	The late Major A. A. Dorrien Smith
About 1 Sept.	Marloes sand, Dale Fort, Pembrokeshire	1	Empty shell	Mr J. H. Barrett

TABLE I (*continued*)

Date	Locality	Number	Condition	Recorder
Sixth week (5–11 Sept.)				
5 Sept.	Carbis Bay	1	Empty shell	Mr A. Hutton
5 Sept.	Braunton Sands, near Barnstaple	Few	Empty shells	Mrs Wiseman
Seventh week (12–18 Sept.)				
12 Sept.	Sennen Cove	1	Living	Mr and Mrs H. H. Sheldon

specimens and many more were broken. She enclosed the remains of a shell to confirm the identification. Dr J. R. Bruce found one living *Ianthina* sp. washed up in White Park Bay, Co. Antrim, early in July, along with thousands of dead, but fresh, *Velella* skeletons.

It may be assumed therefore that the shoal which stranded in Cornwall in August came in from the Atlantic to the south of Ireland without touching its shores. It seems most unlikely that it was part of the shoal that stranded on Achill Island in May, probably early. The stormiest weather in May was during the first week, and for the rest of that month the winds were mostly below the average.

Records of strandings during the first week of August (see Table I) are scanty. Even though the appeal for information did not appear in the press until 3 weeks later it seems clear that there were indeed few strandings at this time and all were on the Isles of Scilly. One of these was definitely a living mollusc; four of the others were empty shells when found; information is incomplete for the other five. Immediately after the appearance of the press appeal the late Major A. A. Dorrien Smith of Tresco organized a search; only one shell (presumably empty) was found (on Bryher) and was kept by the finder. From these few records it can be inferred that the oncoming shoal, drifting before the wind, almost entirely missed the Isles of Scilly, and passed, in view of what happened later, north of them.

The second week of August brought many strandings on the mainland. Early in the week a living *Ianthina* was found in the Land's End district, near Sennen, and two at Zennor a little farther north; from the middle of the week onwards large numbers were stranded along an extensive length of coastline from Land's End to north Devon. There appear to have been particularly dense concentrations near Bude and in Bideford Bay, but there were also some heavy strandings farther south at Perranporth, Sennen, and on the western shore of the Lizard peninsula. None were recorded at any time farther up the English Channel. The Perranporth record is a little vague as to date, but most probably refers to this period.

During this second week winds were at first more or less westerly, light to moderate, increasing in strength on the 9th to blow strongly from the north-west and continuing fresh from the same quarter on the 10th. Early on the 11th it was a little north of west, later swinging round to the south. It was on the 10th–11th that most strandings seem to have taken place, and it was most probably on the 11th at Gunwalloe. The wind continued light south-westerly over the 12th, becoming almost due west on the 13th and dropping in strength.

Relatively few strandings were recorded during the third week and these were mostly in the Padstow district, and towards the end of the week. Winds were at first light to moderate, variable in direction, freshening on the 17th and 18th, until they were blowing strongly from almost due west, veering later to north and decreasing somewhat. They remained fresh and northerly until the end of the week. Northerly winds blow straight onshore in Trevone and Harlyn Bays, but they would also strand *Ianthina* on other parts of the coast if any were sufficiently close offshore.

During the fourth week there were strandings over a longer length of coastline, that from St Ives Bay to Woolacombe. The single record from the Isles of Scilly was of an empty shell which had undoubtedly stranded earlier. Most of the records are of living specimens, never in any great number. The largest number recorded was from near Hayle on 25 August, but an unknown proportion of these were empty and must have been from earlier strandings. Until the 25th the winds were north and north-westerly, mainly moderate; they died away on the 26th, and on the 27th and 28th were light westerly veering north.

For the fifth week the only living mollusc recorded was from Constantine Bay on the first day of the week. Thereafter only empty shells, or presumed empty shells, were found. These were most probably from earlier strandings; and although a living specimen was found at Sennen on 12 September, the invasion was over. That this was due to absence in the area of further living specimens and not to the wind is certain. The week had opened with light or moderate winds from the west, veering to the south-east on 31 August and 1 September. They were moderate and mainly from the south-west on 2 September, increasing in strength and becoming westerly and strong on the 3rd. On 4 September there was a calm. Light to fresh rather southerly winds followed, becoming westerly. On 9 September it blew strongly from the west and remained fresh and strong until the 12th, on which day the living specimen was found at Sennen. Thereafter, for the remainder of the month winds were often strong from a westerly direction, the total run of the winds being substantially above the average. No more *Ianthina* came in, but they were followed from mid-September onwards by large numbers of *Physalia physalis*, which was also abundant on the coast of southern England.

To sum up: excepting for the one notable find of an empty shell at Dale Fort in Pembrokeshire, the strandings were confined to the north coasts of Cornwall and Devon and, in the south, the west coast of the Lizard peninsula. The shoal, or shoals, blown in from the west, missed southern Ireland and barely touched the Isles of Scilly; the main strandings were in the second week of August during a time of fresh or strong north-westerly winds.

During the third week more, but apparently smaller numbers, came ashore in the middle of the region at a time of northerly winds. Thereafter, the number of living animals found quickly declined and one only was taken at Sennen after the end of August.

Some Previous Strandings in South-west England and Wales

It is known that three species of *Ianthina*—in current nomenclature *I. exigua* Lamarck, *I. pallida* Thompson, *I. janthina* (L.)—strand from time to time in south-west England and Wales (Forbes & Hanley, 1853; Fowler, 1947, 1948, 1949; Graham, 1954) as well as in Ireland and Scotland. Mr T. G. W. Fowler, who has searched the beaches at Sennen regularly for many years, tells us, in a personal letter, that the 1954 specimens were 'the first for quite two years'. He remarks that he knows 'there were a few at Constantine Bay in September 1937 or '38'. The main interest in the 1954 strandings is the unusually long length of coastline affected, and probably the numbers stranded were, for that locality, far greater than for very many years. Some correspondents who live on or visit the north Cornish coast and regularly search the beaches there assured us that they had never seen these shells before. We ourselves on numerous visits to the Padstow district from about 1933 had never encountered them. A few older people remembered having seen some washed up about fifty years previously. Mr C. Roberts of Trevone told us he had seen similar shells at Harlyn Bay in 1903 or 1904. Mrs Doris Wilson, writing from Braunton, remarked that her friend 'Mrs Wiseman had found some, only much smaller specimens, about fifty years ago in much the same place' (at Braunton). Miss E. M. Ferguson, writing from Somerset, mentioned finding six empty *Ianthina* shells at Croyde in North Devon, in 1903 she thinks. There are thus three quite independent witnesses to a stranding about that time, and fairly good evidence that there has not been an extensive stranding of *Ianthina* along the north coasts of Cornwall and Devon since then.

Mr J. H. Barrett, writing from Dale Fort Field Centre, Pembrokeshire, notified us that about a dozen dead shells and one live specimen were found at West Dale on 6 September 1950. Another dead, but with float, was found on Marloes sands on 20 August 1953. All were identified as *I. britannica* (= *I. janthina*).

Coincident Strandings of *Velella*

One of the main foods of *Ianthina* is *Velella*, and it was to be expected that this latter organism would strand with it. We ourselves found a considerable number of living and recently dead *Velella* stranded at Trevone and Harlyn Bay at the same time. Mr T. G. W. Fowler reported to us 'hundreds and thousands of live *Velella*' at about the same time as the *Ianthina* strandings at Sennen. The Rev. P. H. T. Hartley saw many living *Velella* on Gwenver beach on 8 August, and stated that they were 'at a density of about one to the yard of tide edge'. Mrs Doris Wilson also saw a few at Saunton at the same time as *Ianthina*. Mr S. R. Nutman had seen many fresh skeletons, one with remains of living tissues, on 30 July at Polzeath. During the first few days of August a small number of living ones were stranded at Wembury near Plymouth. No doubt *Velella* were seen elsewhere but not reported; in order to avoid confusion no mention of *Velella* was made in the letters to the press.

Shell Shape and Growth

The very extensive and confusing synonymy of the genus has recently been clarified by Laursen (1953). Of the many species of *Ianthina* described he recognizes only five, of which only *I. janthina* is viviparous. He figures variations in shell shape, showing how two series diverge from the two large specimens which, of the four shells in the Linnean collection, he regards as representing the type. At one extreme are trochoid shells (narrow in proportion to height), at the other they are flattened (wide in proportion to height). It was on the basis of these varied shapes alone that so many species were described; anatomically they are similar.

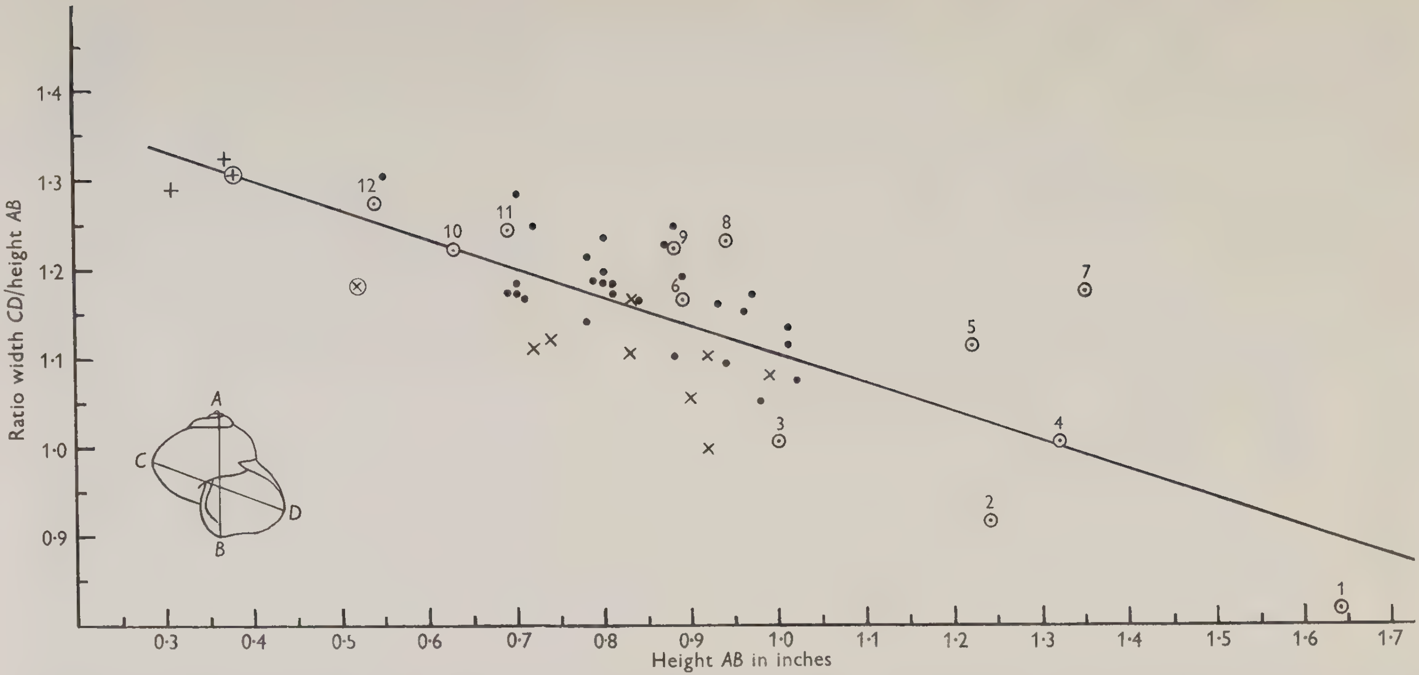
Although among the 1954 specimens there was a considerable range of shell shape it was clear that all intermediate varieties between the extremes were present and that only one species was involved. The majority of these fragile shells were damaged but there were a fair number unbroken. Their heights and widths were measured with calipers graduated in hundredths of an inch. The width recorded was the maximum distance across the last whorl and is inclined at an angle to the height (Text-fig. 2). These measurements are recorded in Table II. No broken shell was larger or smaller. In the graph (Text-fig. 2) the height is plotted against the ratio width/height, and it will be seen that in spite of much variation between individuals of similar heights the general tendency is for the ratio to get smaller as the shell grows. Mr G. M. Spooner has kindly estimated the regression of width on height and finds that this tendency is significant. The line drawn through the points in Text-fig. 2 represents the best fit, assuming a linear relation between the ratio and height, through the plots of the thirty-eight observations listed in Table II. It will be seen that the additional points also fall on either side of this line, including the projected portion beyond a height of 1·1. Wide flattened types (= *I. planispirata* Adams & Reeve) are commoner among the smaller shells than the larger. The only shell in which height and width were equal came from Saunton, and it is interesting that four of the eight measurable shells from that district were somewhat taller types than those from elsewhere. This may be without significance; on the other hand, the shells stranded at Saunton may have come from the borders of the shoal, for *Ianthina* was not reported farther east along the coast.

The diagrams of shells given by Laursen in his fig. 15 (1953) have been carefully drawn and are reproduced natural size. Nos. 3, 7 and 12 are from the original material of Linnaeus. These diagrams have been measured with the calipers and the results plotted in Text-fig. 2, along with those for the 1954 shells. It will be seen that they lie about the same curve, though it is noteworthy that the large and relatively broad shell no. 7 (identical with the type from the Linnean collection) falls farther to one side of it than do any of the others. Also plotted are measurements of two shells of *I. planispirata* Adams & Reeve, collected by Mr T. G. W. Fowler near Sennen and sent to us some years ago; his figures of a similar shell (1946) and one of *I. britannica* Forbes & Hanley (1948) from near Sennen Cove, were measured with calipers, and the results, adjusted according to the scale given on the plates, are also plotted.

TABLE II. MEASUREMENTS OF SHELLS OF *IANTHINA JANTHINA* (L.)

Height (in.)	Width (in.)	Ratio: width/height
Shells from Trevone and Harlyn Bays, 1954		
0·55	0·72	1·309
0·69	0·81	1·174
0·70	0·83	1·186
0·70	0·90	1·286
0·71	0·83	1·169
0·72	0·90	1·250
0·78	0·95	1·218
0·78	0·89	1·141
0·79	0·94	1·190
0·80	0·95	1·188
0·80	0·99	1·238
0·81	0·96	1·185
0·81	0·95	1·173
0·84	0·98	1·167
0·87	1·07	1·230
0·88	1·10	1·250
0·89	1·06	1·191
0·93	1·08	1·161
0·94	1·03	1·096
0·96	1·11	1·156
0·97	1·13	1·165
0·98	1·03	1·051
1·01	1·12	1·109
1·01	1·15	1·139
1·02	1·10	1·078
Shell from Porthcothan, 1954		
0·70	0·82	1·171
Shell from Lelant, 1954		
0·80	0·96	1·200
Shell from St Agnes, 1954		
0·88	0·97	1·102
Shells from Saunton, 1954		
0·72	0·80	1·111
0·74	0·83	1·122
0·83	0·92	1·108
0·83	0·97	1·169
0·90	0·95	1·056
0·92	0·92	1·000
0·92	1·03	1·120
0·99	1·07	1·081
Shells sent from Sennen by Mr T. G. W. Fowler some years ago (*planispirata* variety)		
0·31	0·40	1·290
0·37	0·49	1·324

Measurements of the 1954 shells thus confirm Laursen's conclusion, based on comparative evidence, that these varied types belong to one species. He was unable to explain their origin, suggesting that it might be related to variations in shell shape at the veliger stage brought about by differing spatial conditions during embryonic growth. Measurements of veliger larvae obtained



Text-fig. 2. Graph to illustrate the relation of shell width to shell height in *Ianthina janthina*, expressed as ratio of width to height, based on specimens and drawings. The diagram at the side indicates the shell dimensions measured: *AB* the height, *CD* the width. Shells collected in August 1954 at Trevone, Harlyn Bay, Porthcothan, Lelant and St Agnes are indicated by dots; shells collected the same month at Saunton are shown by ×'s. A dot within a circle with a number over it indicates a similarly numbered drawing in Laursen (1953, fig. 15). Two shells of the synonymous *I. planispirata* collected by Mr T. G. W. Fowler at Sennen prior to 1954 are shown by +'s. A similar cross in a circle indicates Fowler's *I. planispirata* from *J. Conch.*, Vol. 22, p. 186, plate I. A × in a circle indicates Fowler's *I. britannica* from the same volume, p. 308. For explanation of the line see text, p. 297.

in 1954 (see below) gave ratios width/height between 1·54 and 1·75. Veligers are thus considerably broader relative to height than any of the shells plotted in Text-fig. 2.

THE FLOAT

The size of the float relative to that of the animal varied greatly, sometimes being quite large with the older portion ragged and worn as in Pl. I, fig. 1, in others smaller and without a degenerating end as in Pl. I, figs. 2 and 3. Mr Peter David regards the degenerating end as atypical, and informs us that in the hundred or so specimens he has taken at sea while in *Discovery II* he has never seen such a thing, and he wonders if it is due to an unfavourable environment. Laursen (1953) figures the curved float of *I. exigua*, and shows such an end, remarking that 'when the parts of the float grow old, they go to pieces and are broken off' (*loc. cit.* p. 6). It may be noted here that while the greater part of the combined animal and float is under water quite a light puff of breath directed at the float will make the snail scud along before it.

A few of the least damaged of our specimens, which had been gathered directly from the sea, added to their floats while kept in dishes of sea water. The method of building has been described several times before, the earliest account being that of Coates (1825). Jeffreys (1867) gives a good account of early observations. Fraenkel (1927) watched a species that appeared at Naples (apparently *I. pallida* Thompson). We proved, as others had done, that an *Ianthina* sunk to the bottom of a vessel by partial destruction of its float is unable to rise to the surface, but that if the water-level be lowered until the propodium is able to break through the water surface, the snail will be afloat again in a few minutes. It is the function of this part of the foot to form mucus-coated bubbles of air and to attach them to the proximal end of the float—briefly, the propodium is stretched upwards, the upper part bends back at right angles and flattens out on the water-surface film, with its intensely black secreting surface upwards, in contact with the air. Its centre is then depressed so that the whole organ becomes spoon-shaped, then hood-shaped; the edges of the 'hood' (Pl. I, fig. 2) contract together, meeting closely enough to prevent water entering the cavity. The propodium is then drawn under the water and the bubble smartly clapped against the base of the float (Pl. I, fig. 3), held in position for a few seconds and then cemented in place by a side-to-side motion of the propodium, wiping over it and presumably spreading additional mucus. Sometimes the new bubble fails to be attached and floats away as a tiny glassy sphere. By pressure the bubbles become polyhedral. The completed float is firm between the fingers, springy and dry—it is not in any sense sticky.

One individual of *I. janthina* formed a bubble in about 65 sec, making perhaps ten in succession and then pausing for a while. Fraenkel's animals took only 30–40 sec over it, but, apart from being another species, at Naples in May they were probably at a higher temperature.

THE EPIPODIUM

When the animal is alive the shell is very slippery. Possibly this may bear some relation to the lateral extension of the foot—the epipodium. As described by Adams (1862), in lively individuals it is 'reflexed on the right side on the penultimate whorl of the shell' (Pl. I, figs. 1–3). The margin is irregularly notched, when fully expanded the right-hand lobe is thin and transparent; only the left-hand lobe is pigmented. Master Richard Wilson noticed rippling movements of the expanded right-hand lobe and suggested a possible connexion with the passage of water over the gills.

Laursen (1953) thinks the epipodium may serve to give the animal a better balance in the water: we saw no sign of its acting as a fin for balance or propulsion, and our specimens never of their own accord moved away from any position where they happened to be floating against the side of the jar or basin, even after many hours. Incidentally, Adams in his 1862 paper did not make the statement attributed to him by Laursen that it co-operated in the animal's movements. It had been made by earlier writers, and the point was discussed by Moerch (1860). Movements of the animal are mainly contractions and extensions of the body, bringing the shell close to the float or about its width lower in the water, and also twisting the body. We had an impression that although *Ianthina* has no eyes the expanded animal when in a glass jar could perceive our near approach and would contract into the shell. Our animals discharged red-violet liquid when handled, but the amount was small. The ink streamed downwards and was soon dispersed.

FEEDING

Ianthina is said to feed on a number of pelagic animals (see Laursen, 1953, p. 14 for brief summary), but appears to subsist mainly on *Velella*. As the adult has, so far as is known, no power of swimming it can only attack its prey when by chance it drifts against it. We introduced our specimens to living, though battered, *Velella* which had come ashore with them, but the *Ianthina* did not eat them even when their snouts were fully expanded, with the forked tentacles erect, and mandibles and teeth exposed as if ready for food.

We are indebted to Mr Peter David for permission to publish the following notes, which he made in 1954 aboard *Discovery II* when watching *I. janthina* collected at Station 3098, 42° 36′ N., 20° 43′ W. The species was identified by Dr J. E. Morton from specimens brought back by Mr David.

> Several large specimens were found with *Velella* tentacles adhering to them and several *Velella* were taken with small *Ianthina* attached to the under surface. Some of these were kept alive for two days, and at the end of this time the *Ianthina* were still quite active, and apparently browsing on the *Velella*; the latter seemed to be lifeless, but from previous experience of *Velella*, it was known that, when dead, their tentacles drop off; the specimens with *Ianthina* on them did not break up in this way.

> It was observed that the *Ianthina* exuded their purple dye periodically (fairly frequently) while feeding, and it seems possible the dye may be used to anaesthetize the *Velella*. Clean *Velella* floats were seen and taken, and may be all that *Ianthina* leaves of its food....The small individuals feeding on *Velella* had no floats in most cases, though in one case a detached float was attached to the rim of the *Velella*; the float would be in the way while the animal was eating, as it seems to be attached to the foot, and the normal gastropod eating habits are used.

Mr David adds that on the November 1954 cruise of *Discovery II* he also observed an unidentified ovigerous species.

To quote from his letter. 'This animal also feeds on *Velella*, but in a different way; instead of abandoning its float and browsing on the under side of the *Velella*, this species is held by surface tension against the rim of *Velella*, and proceeds to chew semicircular pieces from it, in much the same way as a caterpillar does on the edge of a leaf. As far as I could determine no purple was released, but then the *Ianthina* is out of reach of the *Velella* tentacles.'

REPRODUCTION

On not very complete evidence the Ianthinidae are thought to be protandrous hermaphrodites. *I. janthina* is viviparous, the other species attach egg capsules to the under-surface of the float. In Graham's material (1954) of *I. janthina* the males were all markedly smaller than the females. This was in agreement with earlier observations of Ankel (1930) on another species. Laursen (1953), however, could find males and females of the same size in the one collection, and also saw very large males, sterile individuals and hermaphrodites; it would seem that *Ianthina* can pass through more than one breeding cycle.

We had two active specimens gathered from the margin of the sea, which respectively shed male and 'female' genital products. Both were of about the maximum size we saw. In the later part of the day after collection one animal shed singly at intervals at least thirty-six dark brown cylindric pellets, about $\frac{1}{10}$ in. long, and at first thought to be faecal. They fell to the bottom of the container (a white china basin) and quickly disintegrated, releasing a number of fully developed veliger larvae with brownish purple shells, swimming with a bilobed velum. Twenty or more were in each packet, varying from 100 to 230 μ across the shell at its maximum width. They gyrated actively over the bottom, neither moving upwards, nor congregating towards nor away from the light. Fraenkel's veligers (1927) (probably *I. pallida* Thompson) swam evenly in the aquarium, showing neither geotaxis nor phototaxis, although they had eye-spots.

As a prelude to the shedding of a packet the propodium was withdrawn from its normal resting position appressed to the base of the float, and was furled up and twisted from side to side.

Extrusion was so rapid that it could be missed in the momentary shifting of the gaze, so that only once was it seen. Master Richard Wilson was looking down on this animal when from between the bottom of the foot and the gills a 'little bullet' suddenly shot upwards and out over the (morphologically) left-hand side of the shell, that which is not covered over by the epipodium (Pl. I, figs. 2, 3). Several packets were seen shortly after emergence and there was no doubt that they came out from this side.

The organization of larvae into packets which are violently extruded seems not before to have been noted. Laursen (1953), having described the embryoduct as leading to the inside of the mantle cavity some distance from the rectum, says only that 'the embryos now leave the mother animal and swim freely into the ocean'. This only happened in our animal the next day, when it was less active, and a small number of veligers emerged singly at their own pace.

The second animal, which was in another container, several times shed over the left-hand side of the shell a quantity of mucus containing a number of elongated whitish droplets, about the same length as the larval pellets. They fell gently to the bottom. Further observation was impossible without a microscope, but preserved material showed they must have been aggregates of spermatozeugmata. These seem not to have been seen alive in *I. janthina*, but closely resemble those of a species (probably *I. pallida*) described in detail by Ankel (1930). Each is made up of an oligopyrene spermatozoan, whose head is a flat lanceolate plate with a thicker densely granular base from which emerges a tail. To this are attached by their heads so many eupyrene spermatozoa as to give it the appearance of a fox's brush. The spermatozeugma moves forward by means of undulations of the plate's margin and lashing of the eupyrene spermatozoa.

As *Ianthina* has no powers of locomotion the sexes can only meet when accidentally drifted together and as, too, there are no copulatory organs the method of transference of spermatozoa poses a problem. Graham (1954), indeed, finds nothing in the reproductive physiology of *I. janthina* incompatible with a possibility of self-fertilization, while Ankel (1930) only obtained his spermatozeugmata by dissection. Our observation of the actual discharge of aggregates of these elaborate structures supports the view that they are an adaptation enabling spermatozoa to make the relatively long journeys between individuals of a shoal.

Epizoic Animals

On a shell (2·1 cm high) of a living *Ianthina* picked up at Trevone there were growing four living specimens of *Lepas pectinata* Spengler. The capitulum of the largest barnacle was 1·3 cm long, of the smallest 1·1 cm. They were attached in a cluster close to the mouth of the shell beside the columella, and in the normal floating position of the snail the barnacles would be growing more or less upright, pressing perhaps against the base of the float, which was not present when they were found. Over part of the capitulum of one barnacle there was growing a colony of the hydroid *Laomedea geniculata* (L.). Another colony of the same species of hydroid was growing on the shell of the *Ianthina* whose photograph is reproduced in Pl. I, fig. 1. Both colonies of the hydroid were kindly identified by Dr P. L. Kramp, who remarks that the species is particularly inclined to attach itself to floating objects.

Grateful thanks are due to the many who have made this paper possible and whose names are recorded in the text. In addition we should like to thank Professor A. Graham and Dr J. E. Morton for placing their knowledge of the Ianthinidae at our disposal. For our account of the winds we acknowledge our indebtedness to the Daily and Monthly Weather Reports of the Meteorological Office, London.

SUMMARY

1. During the exceptional westerly weather of August 1954 large numbers of *Ianthina janthina* (L.) were found on the north coasts of Cornwall and Devon, in what was almost certainly the largest stranding of this species on English shores for fifty years. The strandings are clearly correlated with periods of west to north winds.

2. With the *Ianthina* there were often stranded many living *Velella*.

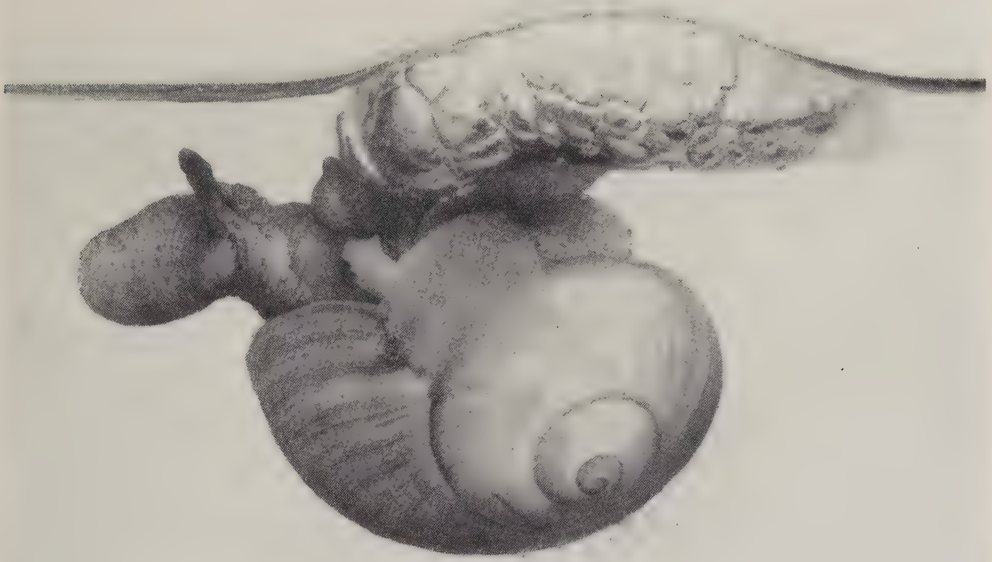
3. A sufficiently large number of unbroken shells were obtained to allow of some investigation into the relationship between width and height. It was found that while there is considerable variation at any one height the width is relatively less in older shells than in young ones. In the past these varied shapes have led to many different species being described. The results in the main confirm Laursen's conclusions (1953); when his careful drawings of different shell types were measured, it was seen that their ratios were in general agreement with those of the 1954 specimens.

4. Earlier accounts of float-building were confirmed from observations of healthy specimens obtained in 1954; a few new details were added.

5. The epipodium as observed in life is described and its function discussed.

6. Some notes on the feeding habits of this species, made by Mr Peter David on board *Discovery II*, are reproduced by permission. He makes the interesting suggestion that the purple dye of *Ianthina* can anaesthetize its main prey, *Velella*.

7. Shedding of larvae and sperms was observed in living specimens. In both sexes the genital products, organized in packets, were shot out of the mouth of the shell on the morphologically left side, that which is not covered by the epipodium. Our observations support the view that the elaborate organization of the sperms into compound structures is an adaptation enabling them to travel from males to females floating well apart.



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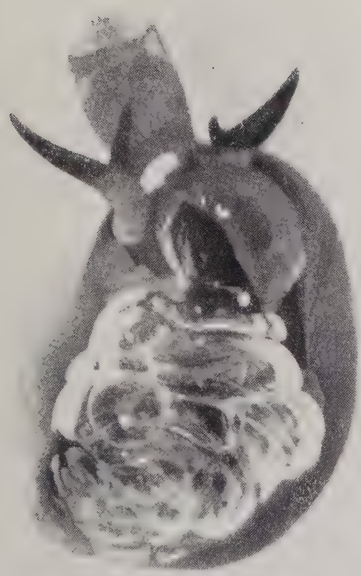

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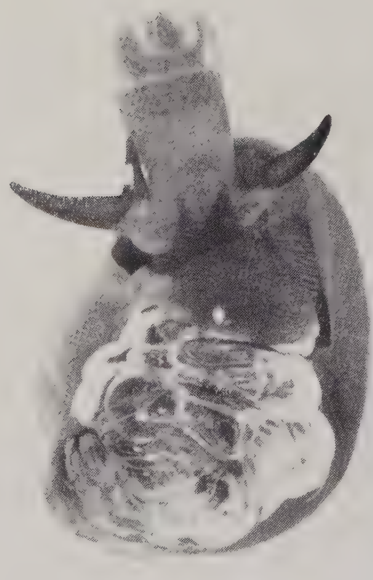

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(*Facing p.* 304)

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EXPLANATION OF PLATE I

(Black and white photographs from Kodachrome originals, taken by electronic flash. Reproduced about twice natural size.)

Fig. 1. Lateral view of a living *Ianthina janthina* L. floating against the side of a cylindrical vessel: the meniscus is arched where the float contacts the glass. The snout and tentacles are not quite fully extended. The colourless translucent epipodium of the right side and its serrated border are well shown. The propodium in its contracted state is seen at rest against the proximal (anterior) end of the float. The distal extremity of the float is disintegrating and losing air. A hydroid is growing on the shell.

Figs. 2, 3. Views from above of another specimen adding a bubble to its float. In Fig. 2 the edges of the expanded hood-shaped propodium are contracting together to enclose a bubble of air. Within the hood is visible the dark epithelium, which secretes a coat of mucus around the bubble. In Fig. 3 the propodium is pressing the bubble against the base of the float and cementing it into position. In these photographs the snout and tentacles are fully extended. The irregular white patches are highlights where wet, and therefore shiny, rounded parts just break the surface film. In this view only the top of the bubble float, which is entirely above water, is visible. The epipodium of the right side (left side of each picture) is seen wrapping around the shell surface. Gill lamellae within the mantle cavity are glimpsed through the shell mouth, especially in Fig. 3. It is through this open portion of the shell mouth, on the left side of the snail, that the genital products were shed (see page 303).

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NOTES ON THE BIOLOGY OF *SERTULARIA ARGENTEA* L.

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(Text-figs. 1–5)

A fishery has developed in recent years in this country for certain hydroids collectively termed 'white weed'. The hydroids, particularly *Sertularia* and, more recently, *Hydrallmania*, are raked up from the sea-bed, processed, dyed and used, largely in the United States of America, for decorative purposes. Fishing for white weed is not new, for it was practised in Germany between the wars. German scientists, notably von Reitzenstein (1913), Pax (1928), and Thiel (1938), examined various aspects of the fishery, and of biology of the hydroids concerned.

The main centre of the industry is the Thames Estuary, where the hydroids grow in extensive beds, on a bottom of sand and shells, on which the weed can be fished commercially by boats equipped with simple iron rakes.

TAXONOMY

There has been some difference of opinion on the naming of the various species among the Sertulariidae. The two species concerned in this work were first named by Linnaeus (1758) as *Sertularia cupressina* and *S. argentea*. He subsequently renamed them (1767) *S. cupressina* and *S. cupressina* var. *β argentea*. Since then various authors, including Hincks (1868), have maintained their independence, while others, notably Broch (1918), followed by Kramp (1938) and Leloup (1938), have placed them together as *S. cupressina*. Broch (1918) considered that Hincks's (1868) distinction between the two species was based on characters which show too much variation. He later (1928) referred to them as *S. cupressina* forma *typica* (Broch) and *S. cupressina* forma *argentea* (L.). Von Reitzenstein (1913) followed Hincks (1868), and throughout his description of the hydroid used in the German whiteweed fishery used the name *S. argentea* (Ellis & Solander, 1786). Thiel (1938), however, figured and described the same species as *S. cupressina*, basing his description on that of Broch (1928). Others, including Nutting (1904) and McLean Fraser (1944), have removed them as separate species to the genus *Thuiaria* Fleming, the distinction between *Sertularia* and *Thuiaria* being the arrangement of the hydrothecae, which are in opposite pairs in *Sertularia* and alternate in *Thuiaria*.

It is not proposed to discuss the case for a change of genus in this paper, but it is important to diagnose correctly the organisms concerned in the whiteweed fishery, particularly when referring to aspects of their biology and ecology.

The hydroid occurring in great quantity in the Thames Estuary, and used for preference in the whiteweed industry, is *Sertularia argentea* L. = *S. argentea* Ellis & Solander sec. Hincks (1868). This is easily distinguishable from *Sertularia cupressina* L. sec. Hincks (1868), and there seems to be no good reason why they should be considered to be the same species. Since this paper is mainly concerned with only one of these two species, this seems to be a good opportunity to emphasize their outstanding differences.

In the Thames Estuary, although most of the weed is normal *S. argentea*, there appears with it, in smaller proportion, a distinct form which has an altogether more luxuriant bushy growth. This form, which we shall call for convenience the 'bushy type', is easily distinguished by fishermen, and is less acceptable to the processers, who prefer to have many colonies per unit weight. The fresh weed is bought from the fishermen by weight and sold, when processed, as bunches of colonies. This bushy form shows no anatomical differences from the normal type, with the exception that the side branches are more subdivided and greatly elongated, as though the colony has been subjected to better growth conditions (Fig. 1 F, K). The proportion of bushy type *Sertularia* is small in winter and spring, but larger in summer and early autumn; it may therefore be a more luxuriant growth form produced from the normal type under summer conditions. However, in early winter, when much less of this type is found, long colonies of the normal-type *Sertularia* are still much in evidence, with no signs of any previous extension of their side branches. At the growing point the structure of the bushy form is identical with that of the typical *S. argentea*. Reference to Fig. 1 will leave little doubt that these are merely growth forms of the same species.

In North Wales there is another Sertularian, so far not observed in the Thames Estuary, which agrees closely with Hincks's (1868) account of *S. cupressina*. Superficially, in growth form, it resembles the bushy type of *S. argentea*, and is probably less suitable for commercial purposes. It is characteristic of fairly exposed sand-banks off the North Wales coast, and with it occurs, in smaller quantity, some *S. argentea*, mostly of the bushy type. In the Menai Straits, towards the eastern end, *S. argentea*, normal type, occurs predominantly with only a little *S. cupressina*. All three forms have been separated easily by untrained fishermen in North Wales.

In Fig. 1 are compared the growth forms of the two species, which differ markedly. The woodcuts in Hincks are excellent representations of the two species, but in all the specimens we have seen, the apex of *S. argentea* has been more tapering than that of *S. cupressina* (Figs. 1 A, E; 2). This could be attributed to variation in growth rates at different times between the two

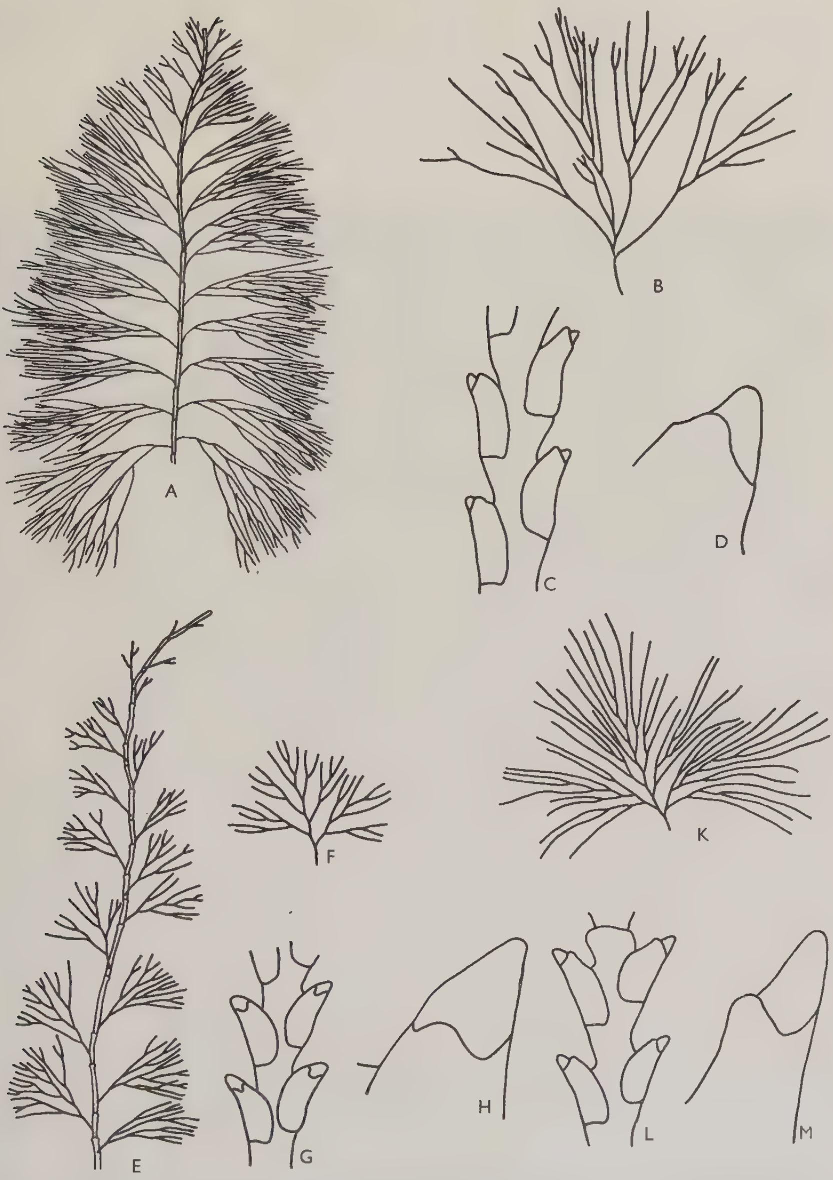

Fig. 1. Outline drawings showing the morphology of *Sertularia*. A–D, *S. cupressina*, North Wales; E–H, *S. argentea* (normal type), Thames Estuary; K–M, *S. argentea* (bushy type), Thames Estuary. A, E, show the arrangement of the side branches on the main axis, $\times \frac{3}{4}$; B, F, K, the subdivision of the side branches, $\times 1\frac{1}{4}$; C, G, L, the hydrothecae on the side branches, × 25 and D, H, M, the hydrothecal margins, × 125.

species. A very important difference is the arrangement of the branches, which arise in a definite spiral fashion from all sides of the stem in *S. argentea*, while in *S. cupressina* they are almost strictly alternate and in one plane, with only the faintest spiralling of the main stem towards the apex of the colony. This characteristic formed the basis of McLean Fraser's (1944) separation of

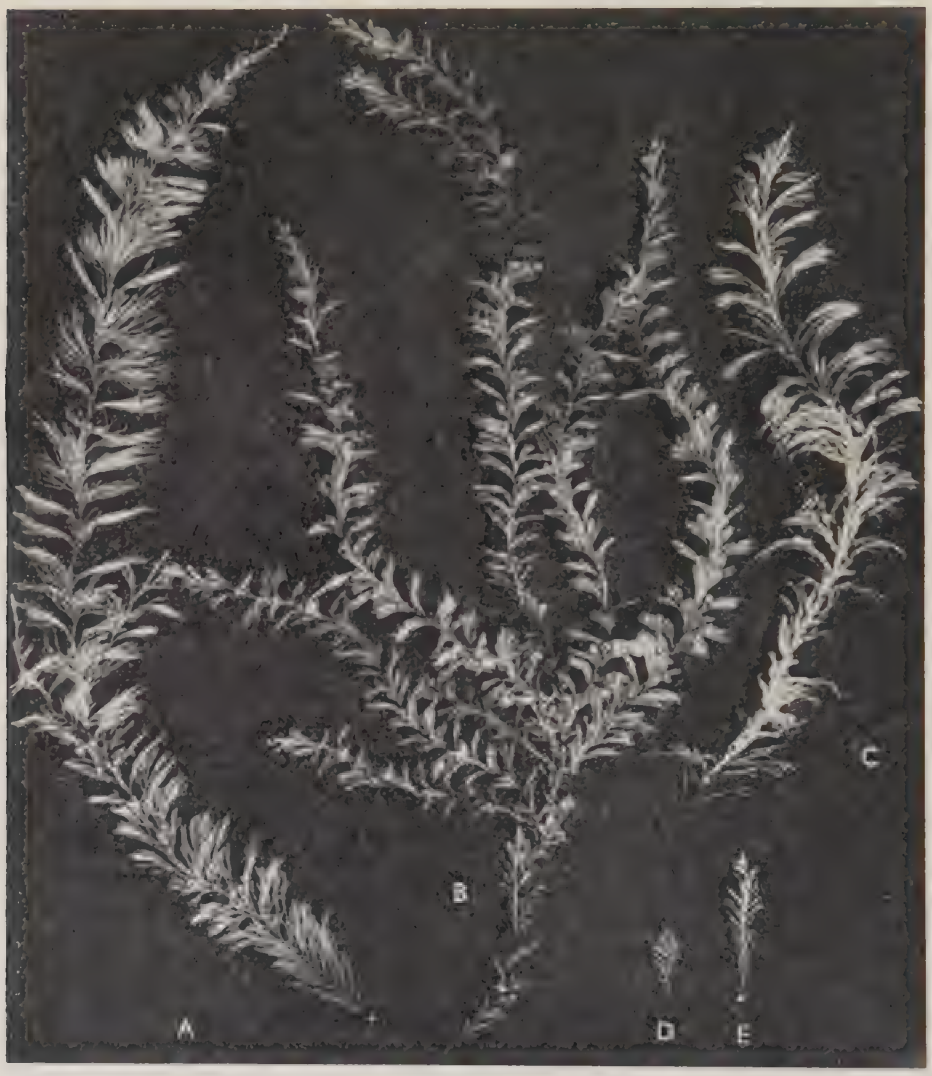

Fig. 2. A, *S. argentea*, bushy type, Thames Estuary; B, *S. argentea*, normal type, Thames Estuary; C, *S. cupressina*, North Wales; D, E, *S. argentea*, two young colonies. × ½. (Photo: R. Elms.)

the two species. Broch (1928) was also aware of it when he described the two forms of *S. cupressina*. In *S. cupressina* the internodes are well defined, each bearing two opposite branches, rarely one or three, and a pair of opposite hydrothecae. Variations from two branches per internode can usually be explained by previous breakage or change in direction of growth of the colony. In *S. argentea*, each internode of the stem bears only one branch, rarely two, and several, usually two or three, opposite pairs of hydrothecae. It is interesting to note that in the young forms of *S. argentea*, on the first few centimetres of stem, the branching is in one plane, with usually two branches to each internode as in *S. cupressina* (Fig. 2). In Hincks's and von Reitzenstein's accounts, *S. argentea* is said to have two branches to each internode, but they may have been referring to the older part of the colony near the base; the branches are subdivided by regular dichotomy (Fig. 1 F), and stand out rigidly from the stem. The side branches of *S. cupressina* are less regularly subdivided, and more elongated, giving the whole colony a more graceful appearance. The elongation of the side branches of *S. argentea*, bushy form, is responsible for the superficial likeness between it and *S. cupressina*. The slenderness of the pinnules in *S. cupressina* is emphasized by the position of the hydrothecae, which are alternate and each is adnate throughout most of its length (Fig. 1 C). The apices of the hydrothecae are less divergent than in *S. argentea*. The hydrothecae of *S. cupressina* have a more open alternate arrangement, whereas in *S. argentea* it would be more correct to describe the hydrothecae as subopposite (Fig. 1 G). In *S. cupressina* the apex of each hydrotheca is produced into two almost equal teeth, but in *S. argentea*, the teeth are unequal (Fig. 1 D, H, M).

It is agreed with Broch (1918) that slight variations in the arrangement and form of the hydrothecae are found in a single colony, particularly between parts of different age, and for this reason less importance has been attached to them in the separation of the two species. The differences described were, however, evident when examining strictly comparable parts of colonies of similar size of the two species.

These differences in the characters of the two species are sufficient for easy separation and justify their maintenance as separate species.

For completeness, the characters of the two species are here listed partly after Hincks (1868) and McLean Fraser (1944).

Sertularia argentea L.

Colonies usually up to 30–40 cm, sometimes much longer and greatly branched. Branches arise from all sides of the stem with 'bottle brush' effect, usually one branch to each internode, but in young colonies branching in one plane, two to each internode. Branches subdivided by regular dichotomy; hydrothecae usually subopposite, curved gradually outwards, margin with two teeth, one usually longer than the other; operculum of two flaps.

Sertularia cupressina L.

Colonies usually up to 30–35 cm, occasionally much longer, branches regularly alternate, on opposite sides of stem only, usually two branches to each internode, upper part of stem may be faintly spiralled. Branches subdivided alternately rather than dichotomously; hydrothecae alternate, tubular, largely immersed and not strongly turned outward, margin with two almost equal teeth; operculum of two flaps.

REPRODUCTION

Hincks (1868) and Nutting (1904) have given a general account of reproduction throughout the Sertulariidae. Von Reitzenstein (1913) has described the reproduction of *S. argentea* L. in German waters. Teissier (1923) described the development and structure of the gonangia in *Dynamena pumila* (L.).

The multiplication of polyps to form a colony might equally be considered as growth or asexual reproduction. A second method of asexual reproduction is by the ramification of the stolon over the substrate from the basal disc. When a colony is removed from the substrate it is usually found to be attached to the bases of several others. This condition could result from planulae settling among the stolon extensions of one hydroid, or from the uniting of runners from several. It can, however, be seen to result, at least in part, from the formation of small colonies at the tips of the runners. (When colonies are encrusted by other organisms treatment with acid helps to reveal details of structure.) Von Reitzenstein (1913), too, suggested that this might be a form of asexual reproduction, exhibited most often at the end of the sexual period.

The most important form of reproduction is sexual. Vase-shaped gonangia, slightly flattened in a plane at right angles to the direction of the branch, are produced from the side branches, nearly perpendicular to the plane of the hydrothecae (Fig. 3). The fully developed gonangium is about 1 mm in length. The branches bearing gonangia commence at a variable distance from the base, depending on the size and age of the colony, and extend to within a few cm of the apex. On an established breeding colony the youngest gonangia are found on the uppermost branches and towards the tips of those lower down. The most mature gonangia are usually more distant from the apex.

Thiel (1938) followed van Beneden (1866) and Goette's (1907) descriptions of the development of the female gonangium. He did not, however, describe the development of the male sex products, and disregarded von Reitzenstein's (1913) figure of the male gonotheca as being without foundation or description. Thiel's (1938) dogmatic statements, based, it seems, on the observations of other workers, concerning von Reitzenstein's work on breeding, are misleading, and it was not until after much of this work had been completed that it was found that several of von Reitzenstein's observations had been confirmed.

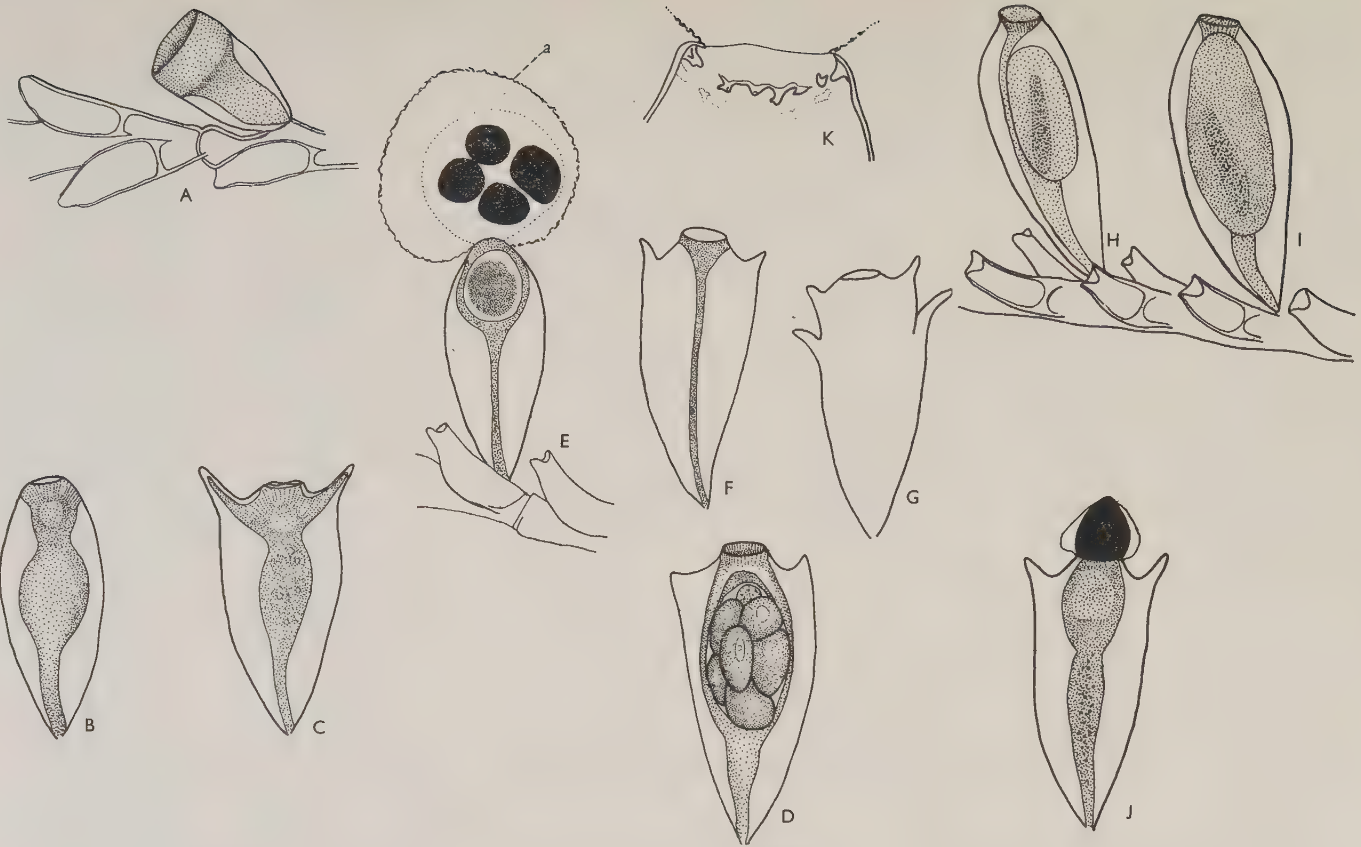

Fig. 3. Stages in development of the gonangium of *S. argentea*. A–G, female gonangia, × 33; H–J, male gonangia, × 33; K, terminal aperture of female gonangium, × 115. *a*, acrocyst.

The development and structure of the male and female gonangia are being studied in detail and will provide the subject of another paper. It is here intended merely to point out the gross differences between the male and female gonangia in their various stages of development, as an aid to their identification. In both sexes the gonotheca arises from the side branch as a small cup-shaped outgrowth (Fig. 3 A), which gradually elongates and closes to give the typical form (Fig. 3 C). There may be one to four, but usually two, lateral horns flanking the circular terminal aperture (Fig. 3 G). Inside the aperture there is a series of, usually twelve, minute teeth (Fig. 3 K). In the female gonangium, eggs are produced in a marsupium, which when ripe is extruded as an acrocyst inside which the eggs develop. An acrocyst usually contains

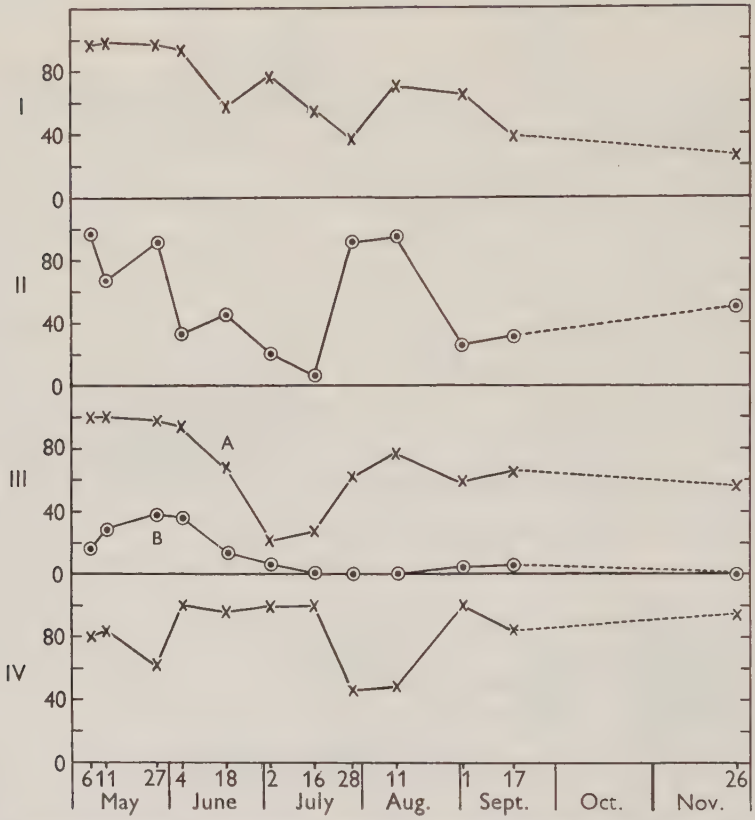

Fig. 4. Graph showing the breeding of *S. argentea* at the Maplin Sands, Thames Estuary, in 1953. I shows the colonies bearing gonangia, expressed as a percentage of the total colonies in a sample; II–IV show the colonies bearing gonangia—II developing, III A ripe, B with female acrocysts; and IV degenerating—expressed as a percentage of the colonies with gonangia.

from two to ten eggs which develop into planulae. Rupture of the acrocyst allows the escape of the planulae (Fig. 5B).

The stages of development of a gonangium are distinguishable (Fig. 3) in fresh material under a microscope. Initially the contents are restricted to an only slightly swollen longitudinal band, enlarged terminally to fill the horns and apex of the gonotheca. Subsequently, the gonotheca becomes full when nearly ripe. In degenerating stages the gonothecae are empty, or contain a narrow strip of tissue slightly enlarged near the aperture. The ripe female gonangium is easily recognized by its content of regularly shaped pink ova, or by the presence of an acrocyst containing developing eggs. The ova can be distinguished at an early stage in the development of the female gonangium.

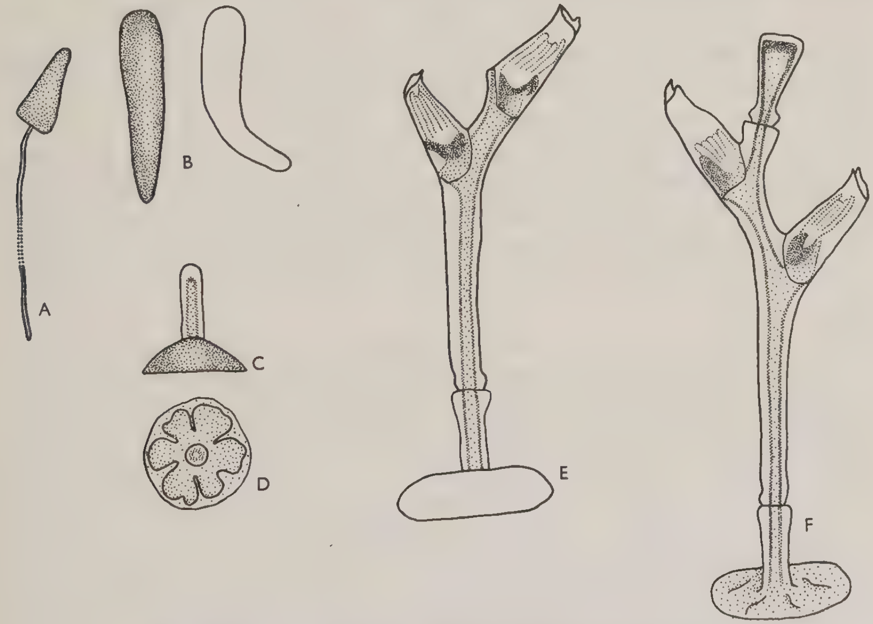

Fig. 5. Early development of *S. argentea*. A, a single sperm; B, planula; C, D, newly settled colony; E, F, after formation of the first few hydrothecae. A, × 4,500; B–F, × 50.

Smears of ripe gonangia from certain colonies carrying developing, mature and degenerating gonangia, but no acrocysts, showed developing and ripe sperm (Fig. 5A). These gonangia were all creamy white, in contrast to the pinkness of the full gonangia of colonies bearing acrocysts. In a study of many colonies, no male gonangium was found on a colony with female acrocysts, nor any female acrocysts on colonies with gonangia containing spermatozoa. It was concluded that there are separate male and female colonies, readily separable when ripe by the colour of the gonangia. Subsequently, the male colonies have been found with a form of acrocyst protruding from the gonangia, but here it is smaller, densely white and full of mature spermatozoa (Fig. 3 J). The male 'acrocyst' has a thin membrane enclosing the sperm, and is less regularly round than that of the female. It seems to be a more transient structure, less likely to be found.

In fresh specimens the sexes can be readily separated by the colour of the ripe gonangia. A further aid to identification of the male is the presence of a dark reddish brown band of pigment, the spadix, which is readily seen in developing male gonangia within a regularly oval translucent sac. This is less easily seen when the ripe gonangium is filled by sperm (Fig. 3 H, I). The degenerating male and female gonangia are very similar. A reddish brown spadix is also present in the female gonangium, but tends to be obscured by the more opaque ova.

There is convincing evidence that each gonangium, male and female, produces more than one liberation of sperm or eggs. This aspect of reproduction will be discussed in a future paper.

The extent of the breeding season was studied by taking random samples of *Sertularia argentea* from dredged catches from the Maplin Sands, Thames Estuary. Only those colonies 70 mm in length and larger, which could be assumed to be capable of breeding, were included within a sample. Shorter colonies were not often taken in the breeding condition on the Maplin Sands.

In each colony the occurrence of developing, ripe and degenerating gonangia was noted. The number of colonies with gonangia has been expressed as a percentage of the total, and the number of colonies with each stage of gonangium is given as a percentage of the total number of colonies with gonangia. The results are shown in Fig. 4. The first sample was taken on 6 May 1953, when there was a high proportion of colonies with developing and ripe gonangia, and slightly less with degenerating gonangia. Evidently the beginning of the breeding season had been missed. By 4 June the number with developing gonangia had dropped, but there were nearly 100% with both ripe and degenerating gonangia. After this date the number with ripe gonangia became less. After a slight increase, the percentage with developmental stages also declined until towards the end of July when a second maximum of colonies with developing gonangia was found. This was followed in mid-August by a peak in the percentage with ripe gonangia and, in early September, by a peak in the number with degenerating gonangia. Although the colonies bearing gonangia formed a high percentage of the total colonies at the time of the first breeding maximum in May/June, their number decreased in July, corresponding with the falling off in the number of colonies with developing and ripe gonangia, and increased only slightly for the second breeding peak in July/August. Thus the second burst of breeding is less intense. Although, in November, the proportions of gonangia-bearing colonies with developing and ripe gonangia were high, yet only 25% of all colonies carried gonangia, so that the total amount of breeding was small.

These observations were made from preserved samples and no attempt was made to distinguish the sexes, but where colonies bearing female acrocysts were present they were noted, and their number expressed as a percentage of the total colonies with gonangia. These reached a maximum in late May. It was later found that the sexes could be quickly distinguished in preserved colonies by dehydrating a mature side branch and examining it in a clearing agent.

To estimate how much earlier than 6 May the breeding season had commenced, ten randomly selected colonies from each of the first three samples were examined more closely, and the stages of the gonangia from every fifth branch counted and recorded. To count and identify the gonangia on every branch would have taken much time; the total number of gonangia on several colonies was therefore counted, and the proportion of each stage compared with the results of counting every fifth branch. This gave a reasonably accurate comparison. The three groups distinguished were: I, early developmental (without horns); II, late developmental and ripe; and III, degenerating; their numbers have been expressed as a percentage of total gonangia in Table I. It seems that the gonangia must first appear during April, but the position of the peak of ripe gonangia at the beginning of June is confirmed (Fig. 4).

TABLE I. THE PERCENTAGES OF THREE STAGES OF GONANGIA IN *SERTULARIA ARGENTEA*

I developing, II ripe, III degenerating, counted on every fifth branch from the colonies in samples from the Maplin Sands in 1953.

	Stage		
Date	I	II	III
6 May	21	41	38
27 May	14	58	28
4 June	1	69	30

The distribution of the various stages in the development and degeneration of the gonangia on a colony was also confirmed by these counts. For example, from the sample of 27 May 1953, a female colony of 420 mm length carried the gonangia shown in Table II, with the youngest gonangia towards the apex and the oldest lower down. Below the eightieth branch from the apex there were a few additional branches, then a region of 60 mm devoid of branches, a usual condition in older colonies.

Von Reitzenstein (1913) found that the breeding period of *S. argentea* in Germany in 1908–9 extended from April to early June, and that when the spawn was released, the empty gonangia fell off. At the beginning of July he could find no more colonies with gonangia; there followed in August a second less important reproductive period when a smaller proportion of colonies carried gonangia. This second breeding period lasted until November when an occasional colony was taken with gonangia, but none in winter.

There is some evidence that the breeding period may vary in length on whiteweed grounds separated by only a few miles. For example, in a sample taken from near West Buxey, about 4 miles from the Maplin Sands, on 25 November 1953, 78% of the colonies bore gonangia, and 28% of the colonies with gonangia carried acrocysts. This was quite a different picture from that obtained on the Maplin Sands (Fig. 4). Occasional colonies with female acrocysts or male gonangia with developing and ripe spermatozoa have been taken throughout the winter months, but the great majority have none.

TABLE II. THE NUMBERS OF GONANGIA ON EACH FIFTH SIDE BRANCH OF A 420 MM COLONY OF *SERTULARIA ARGENTEA*

The stages are I early developmental, II ripe, III degenerating gonangia.

Branch, numbering from apex	Stage		
	I	II	III
0	—	—	—
5	—	—	—
10	—	—	—
15	16	—	—
20	16	78	—
25	9	55	6
30	—	67	1
35	—	61	28
40	—	38	16
45	—	8	10
50	—	13	4
55	—	1	3
60	—	3	5
65	—	1	2
70	—	—	—
75	—	—	—
80	—	—	—

The developing eggs within the acrocysts are about 40–50 μ in diameter. Typically the head of each spermatozoan is an elongated triangle (Fig. 5A), 2–3 μ long and 1–1·5 μ wide, with a tail 30–40 μ long.

DEVELOPMENT

Rupture of the acrocyst allows escape of the ciliated planulae, each of which assumes an elongated form, about 0·5 mm in length, with a somewhat blunt anterior end (Fig. 5B). The planula revolves with a forward swimming, creeping motion. In the laboratory the planulae were not seen to swim near the surface, but remained near the bottom. It seems likely that they are confined to the bottom and are not pelagic. This would increase the tendency for the hydroids to form a dense covering of the substrate, and would lead to the formation of fairly discrete beds, as observed. Hydrographic conditions would clearly exert a considerable influence.

The settlement of the planulae and early development of the colony have been followed in the laboratory. The planula attaches to the substrate, usually a piece of shell or stone, and produces a semi-spherical holdfast—irregularly lobed when viewed from above (Fig. 5C, D). The planulae under observation hatched from acrocysts placed in an aquarium on 8 May 1954. By 11 May they had assumed the form shown in Fig. 5C. This agrees with von Reitzenstein's (1913) estimate of a free swimming period of 2–3 days. By 17 May elongation of the main stem had occurred with a node near the base, and the first two polyps, each fully furnished with a hydrotheca, had been formed. On 20 May, young colonies with three polyps were observed, but after this date development ceased, due to some unfavourable condition, and the young colonies died (Fig. 5E, F). Under laboratory conditions, the initial growth to a length of less than 2 mm took almost 2 weeks. The settlement and early growth of *Dynamena pumila* (L.) have been described by Teissier (1923).

GROWTH

Several attempts have been made to observe the growth rate of *S. argentea* throughout the year in the Thames Estuary. Shells bearing *Sertularia* were dredged, some colonies measured, and the shells attached to concrete blocks which were buoyed, or attached to beacons, and returned to the sea bottom. These experiments were of short duration because the concrete blocks and shells were frequently lost or buried by shifting sand in bad weather. It was found difficult to keep colonies of *Sertularia* alive in the laboratory conditions for long enough to observe growth rates. It is believed that excessive silt in the water supply, due to bad weather, was responsible. The most successful method was to suspend the colonies from a piece of cord stretched across a large tank filled with sea water, shaking the cord periodically to dislodge the silt. The results available from the laboratory, the River Crouch and the Maplin Sands, are given in Table III. In the field quite young colonies were used and the growth rate varied from 0·3 to 1·3 mm per day. This agreed closely with the measurements of growth in the laboratory in which the average increment varied between 0·1 and 1·7 mm per day. An attempt is being made to study the growth rate in the Thames Estuary throughout the year in 1955. The majority of colonies attached to blocks showed negligible growth during February, the maximum recorded being 6 mm in 28 days—that is 0·2 mm per day. During March all but a few colonies showed growth, and a maximum of 0·4 mm per day was recorded.

Von Reitzenstein (1913) found that growth was not continuous throughout the year. The peak of growth (four-fifths of the total) occurred in the months of May to September. His results for *Sertularia* grown in culture boxes in the

TABLE III. GROWTH OF *SERTULARIA ARGENTEA*

All measurements in millimetres.

Date	Length	Remeasured	Growth	Average growth per day	Remeasured	Growth	Average growth per day
RIVER CROUCH							
15 July 1953	28	24 July	6	0·7	14 Aug.	27	1·3
15 July 1953	33	24 July	3	0·3	—	—	—
15 July 1953	58	24 July	5	0·6	—	—	—
15 July 1953	58	24 July	10	1·1	—	—	—
15 July 1953	74	24 July	10	1·1	—	—	—
15 July 1953	78	24 July	4	0·4	—	—	—
MAPLIN SANDS, THAMES ESTUARY							
3 Sept. 1954	68	1 Oct.	0	0	5 Nov.	22	0·6
LABORATORY							
20 Aug. 1953	149	26 Aug.	7	1·2	3 Sept.	1	0·1
20 Aug. 1953	172	26 Aug.	4	0·7	3 Sept.	1	0·1
20 Aug. 1953	206	3 Sept.	10	0·7	—	—	—
20 Aug. 1953	207	26 Aug.	8	1·3	3 Sept.	8	1·0
20 Aug. 1953	236	3 Sept.	2	0·1	—	—	—
20 Aug. 1953	278	3 Sept.	4	0·3	—	—	—
20 Aug. 1953	303	26 Aug.	2	0·3	—	—	—
20 Aug. 1953	352	3 Sept.	24	1·7	—	—	—
4 Aug. 1954	28	10 Aug.	0	0	16 Aug.	2	0·2
4 Aug. 1954	42	10 Aug.	6	1·0	—	—	—
4 Aug. 1954	145	10 Aug.	9	1·5	16 Aug.	4	0·7
4 Aug. 1954	175	10 Aug.	4	0·7	16 Aug.	1	0·2

sea at Hörnum in 1908 may be summarized as follows, all measurements being in mm:

Growth period	Average growth		Maximum growth	
	Over the period	Average per day	Over the period	Average per day
10 Apr.–20 May 1908	3·1	0·05	10·0	0·24
21 May–9 July 1908	12·5	0·25	30·0	0·60
10 July–12 Sept. 1908	50·0	0·77	80·0	1·25
12 Sept.–4 Nov. 1908	26·2	0·50	40·0	0·75
5 Nov.–26 Mar. 1909	10·0	0·07	15·0	0·15

He also cultured *Sertularia* in the sea on a board and on a basket lid, from May to November of the same year. By both these methods the growth rate in the summer months was greater than by the box method. Combining all his results, he arrived at the figure of 228·4 mm average growth and 285·0 mm maximum growth between 10 April 1908 and 26 March 1909.

Von Reitzenstein's growth rates are of the same order as those obtained for the Thames Estuary. The difficulties of obtaining growth measurements have been described; von Reitzenstein's results are open to the criticism that he included many colonies of different sizes which may well have had quite different growth rates, and further, the results were not based on the same colonies over the whole period. Von Reitzenstein himself, however, was aware of the weaknesses in his methods. Thiel (1938), commenting on von Reitzenstein's results, stated that the growth recorded must have been much lower than that occurring under natural conditions, but gave no reasons for this opinion. The maximum growth recorded by von Reitzenstein between 10 July and 12 September 1908 averaged 1·7–1·8 mm per day, which agrees closely with our maximum growth increment which averaged 1·7 mm per day during August to September 1953 under as near as possible natural conditions.

It is felt that von Reitzenstein's estimate of about 26 cm annual growth in the sea is not unreasonable. He also gave an indication of the slowing of growth rate with increase in size of a colony. His conclusion that the largest colonies of 60–70 cm length were 2–3 years old was based on the assumption that large and small colonies have the same rate of growth.

Von Reitzenstein also found that colonies cast off branches and side branches in winter when growth virtually ceased. This process is responsible for the lack of side branches in the lower regions of older colonies. Eichelbaum (1912) found that whole colonies, usually those over 30 cm, became detached in autumn, and concluded that some dispersal of the species was effected when the colonies, covered by gonangia, were carried away by currents.

REGENERATION

From the fishery aspect the question of regeneration is of great importance. In a sample of white weed taken from a commercial rake it was found that only 16% of the colonies had been taken with their basal discs intact. The stems of the remainder had been broken just above the base, some across obviously living white stem, others across the dark thickened lower stem close to the point of attachment, a region which has little appearance of being alive.

Using methods similar to those described on growth studies it was found that colonies cut at all levels and returned to the sea were capable of regeneration, even those cut through the blackened region close to the basal disc. Regeneration of the latter was usually by small white sprouts appearing near the cut ends, while in the remainder a continuation of the terminal growth took place. In July 1953 the rate of regeneration by this method was found to vary between 0·1 and 1·4 mm per day (Table IV).

Since cut blackened bases of *Sertularia* colonies are capable of regenerating in this way, it seems likely that the holdfast of a cut colony can also continue to bud vegetatively from the stolon as described earlier (p. 312).

In order to discover whether side branches lost at the approach of winter are capable of becoming attached to form new colonies, several side branches were detached from a mature colony and attached vertically to a glass plate, held in a laboratory tank with running sea water. The experiment was continued from 1 to 21 September 1954, and although none of the side branches produced a holdfast, most of them had begun growing from the cut end. One had produced two small branches, each with four opposite pairs of hydrothecae, and most of the others were growing in a similar way, but nothing resembling a basal disc or stolon was produced. The experiment ended with the death of the branches, probably caused by excess of silt. Many young colonies have been inspected to see if any had an unusual growth form which might have resulted from the attachment of a side branch, but none was found. It is probable that when the side branches are lost the polyps and tissues have been retracted and that they consist merely of skeletal matter, and would therefore be incapable of settlement. The retraction of tissue into the stolon of *Dynamena* (= *Sertularia*) *pumila* (L.) in autumn and winter, followed by the reforming of the polyps in the old thecae in January and February, has been described by Haddow (1936). It does not seem likely that the shedding of branches of *Sertularia argentea* constitutes a form of asexual reproduction. The experiment, however, demonstrated the remarkable powers of regeneration possessed by these hydroids.

TABLE IV. REGENERATION OF *SERTULARIA ARGENTEA*

All measurements in mm.

Date	Length	Cut to	Remeasured	Growth	Average growth per day	Remeasured	Growth	Average growth per day
RIVER CROUCH								
15 July 1953	43	33	24 July	4	0·4	—	—	—
15 July 1953	45	18	24 July	3	0·3	—	—	—
15 July 1953	48	38	24 July	2	0·2	14 Aug.	22	1·0
15 July 1953	49	38	24 July	1	0·1	—	—	—
15 July 1953	60	38	24 July	2	0·2	—	—	—
15 July 1953	65	38	24 July	1	0·1	14 Aug.	12	0·6
15 July 1953	65	48	24 July	4	0·4	14 Aug.	3	0·1
15 July 1953	75	43	24 July	3	0·3	—	—	—
15 July 1953	80	48	24 July	3	0·3	14 Aug.	23	1·1
15 July 1953	110	63	24 July	4	0·4	—	—	—
15 July 1953	158	70	24 July	3	0·3	—	—	—
15 July 1953	158	108	24 July	1	0·1	—	—	—
15 July 1953	180	158	24 July	13	1·4	—	—	—
15 July 1953	190	178	24 July	11	1·0	—	—	—
MAPLIN SANDS								
3 Sept. 1954	21	13	1 Oct.	13	0·5	5 Nov.	16	0·5
3 Sept. 1954	32	22	1 Oct.	9	0·3	5 Nov.	14	0·4
3 Sept. 1954	63	37	1 Oct.	8	0·3	—	—	—
3 Sept. 1954	69	39	1 Oct.	4	0·1	5 Nov.	8	0·2
3 Sept. 1954	98	48	1 Oct.	29	1·0	—	—	—
3 Sept. 1954	118	33	1 Oct.	8	0·3	5 Nov.	7	0·2

A number of colonies, both male and female, have been found bearing gonangia each with a small vegetative branchlet emerging from the mouth. Sometimes this new growth was branched several times, but more usually there was a simple stem with three or four hydrothecae. Dissection showed that in some the theca of each branchlet was continuous with the gonotheca, which contained an extension of the coenosarc. In others the new branch emerged from inside the gonotheca. The arrangement of hydrothecae on the new branches was found to be more like those of the remainder of the colony, than of a newly settled form. Each gonotheca was of adult dimensions, with horns, and contained an extension of the coenosarc continuous with that of the new growth. It seemed likely either that in the course of degeneration of the gonangium the coenosarc had grown through the gonotheca, or that the gonangium had aborted during its development and reverted to vegetative growth. The result was a number of small branchlets in the same plane as the gonangia, that is perpendicular to the normal direction of growth.

COMMENSALS AND PREDATORS

Sertularia and other hydroids provide a satisfactory habitat for a number of free and fixed epizoic forms. Most often associated with *Sertularia* are encrusting bryozoans, particularly *Membranipora pilosa* (L.), which may cover stems, branches and even gonothecae, obviously in some cases with harmful effects. One colony of length 510 mm was found to be covered from 70 to 410 mm from its base by the bryozoan, and another of 280 mm was covered from 40 to 240 mm. Up to 85% of the colonies in a sample of white weed taken from the Maplin Sands in August 1953 carried encrusting Bryozoa. A thickly encrusted main stem is often devoid of side branches, and shows the effects of severe competition and suffocation. The settlement of Bryozoa reduces the commercial value of white weed.

To a lesser extent encrustation by other hydroids such as *Clytia johnstoni* Alder, *Tubularia* sp. and *Obelia* spp. also occurs. Where present they often form a dense covering as do some peritrichous ciliates.

The larvae of several bivalves find a suitable settling place in the fronds of white weed, which may be found with many thousands of mytilid spat on their branches. *Sertularia* taken from Fleetwood in August 1954 was in such a condition, and these accumulations of developing bivalves must be of great interest to demersal fish. From the Maplin Sands samples the largest numbers of bivalve spat were taken in July.

Sertularia also provides a home for a number of free-living forms, in particular, caprellids and pycnogonids. *Caprella linearis* (L.) may occur in abundance, up to an average of five per colony being taken on the Maplin Sands in July 1953. *Nymphon brevirostris* Hodge occurs frequently, and it and the caprellids are hard to distinguish from the *Sertularia* because of their similar colouring. The nudibranch *Idulia coronata* (Gmel.) is frequently taken with its spawn.

It is questionable whether any of these animals feed directly on *Sertularia*. At certain times of the year the lower parts of the older colonies are devoid of branches. This might be due to the feeding of some predator. However, denuding of side branches is almost always confined to the lower part of the stem, and is more likely to be due to the annual decline of the colony towards the end of the year when the lower branches are shed. This has been described by von Reitzenstein (1913).

Colonies have also been taken regularly in which only the bases of certain side branches remained, covered by Bryozoa, while other branches were intact.

Where a bryozoan was present at the base the rest of the branch was dead. This would also contribute towards the loss of side branches.

Of twenty flatfish taken from a whiteweed bed at Fleetwood in August 1954, only two dabs contained isolated fragments of *Sertularia*, which were amongst vast quantities of mussel spat in the stomach. The heavy settlement of mussel spat on *Sertularia* has been mentioned previously and the presence of the *Sertularia* was believed to be incidental.

Hunt (1925) took fragments of hydroids from the stomach of *Leander*, and Mistakidis (in press) has recorded occasional fragments of *Sertularia* from *Pandalus*.

Harrison (1944) mentions that several observers have maintained that caprellids feed on hydroids and algae, but that such an occurrence is probably exceptional. He found that caprellids fed on copepods and nauplii from the plankton. During frequent observations made by the writers caprellids were observed feeding in the manner suggested by Harrison and did not take *Sertularia*.

Idulia has not been observed feeding, but Browne (1907) has described the intensive feeding of *Tergipes* on *Syncoryne*.

It is considered unlikely that the exploitation of white weed can be substantially detrimental to commercial fisheries, while many fishermen believe that the constant harrowing of the sea-bed by the rakes is beneficial to the development of benthos generally, and of assistance to many fish during feeding.

SUMMARY

There has been considerable divergence of opinion on the naming of species of *Sertularia*. It is suggested that Linnaeus's (1758) original names *S. cupressina* and *S. argentea* relate to distinct species, which may be separated on certain anatomical characters, notably the manner of branching. The 'white weed' fished commercially in the Thames Estuary is *S. argentea*.

A study of the reproduction of *S. argentea* in the Thames Estuary showed that separate male and female colonies exist, and a general description of the development of the gonangia in both sexes is given. The main sexual breeding occurs in May and June, with a second, but less intense, period in July/August. The early growth, following settlement of planulae, was observed in the laboratory.

Measurements of growth were made in the field and laboratory. Growth is seasonal, occurring mostly in the summer months. A maximum growth of 1·7 mm per day was recorded.

Examination of natural and experimental material showed that regeneration of cut stems is possible at all levels, and that even detached side branches can continue to grow to form new branches.

The main commensals and predators of *S. argentea* are mentioned with their possible effects on the host and the whiteweed industry.

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STUDIES ON THE BIOLOGY OF LIMPETS

III. HERMAPHRODITISM IN THE THREE BRITISH SPECIES OF *PATELLA*

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(Plate I)

Sex phenomena in molluscs have been widely investigated, and the relevant literature on the subject has been reviewed by Coe (1943, 1944). Coe recognizes ambisexuality (monoecism, hermaphroditism) and unisexuality (dioecism, gonochorism) and further subdivides ambisexuality into functional ambisexuality (functional hermaphroditism), consecutive sexuality, rhythmical consecutive sexuality and alternative sexuality. Of these, functional hermaphroditism is of widespread occurrence: it is encountered in all the main groups, with the exception of scaphopods and cephalopods. Of the more than 20,000 species of living gastropods approximately half are functional hermaphrodites when fully adult (Coe, 1944). Limpets of the genus *Patella* are unisexual, though there is evidence that sex change may occur in some of the species (Orton, 1920, 1928, in *Patella vulgata*; Bacci, 1947, in *P. coerulea*). Little is known with certainty of the mechanism of sex-determination in these animals, but sexuality appears to be labile since it seems that more than 90% of individuals of *P. vulgata* change sex at some stage in their life history. Aberrant sexual forms might therefore reasonably be expected to occur. In the present paper thirty hermaphrodite gonads encountered in an examination of 64,576 limpets are described and their significance in the wider context of sexual phenomena in molluscs is discussed.

The late Prof. J. H. Orton suggested this study and collected most of the hermaphrodite gonads with which it deals. I am most grateful to Dr A. J. Southward for checking Orton's records of hermaphroditism and sex-change in *Patella*, to Dr Margaret Lang and my wife for invaluable assistance with the histological work, and to Mr D. R. R. Burt who identified the cysts of *Ophryocotyle*.

Material and Methods

The series of hermaphrodite gonads described here contains examples from each of the three British species of *Patella*. The specimens were obtained between December 1945, and December 1948, either by the late Prof. J. H. Orton during his routine examination of populations of limpets for breeding data, or by myself. They were collected from widely separated stations round the British coast. All specimens were fixed in Bouin's fluid in sea water and stored in 70% alcohol. Prior to dehydration and embedding, each gonad was drawn and described in ventral surface view. The gonad was then removed from the visceral mass, divided along its longer and shorter axes into four equal parts, and the dorsal and cut surfaces of each part also drawn and described. Serial sections were cut at either 5 or 15 μ thickness, and the sections stained with Mallory's triple stain or Heidenhain's haematoxylin with eosin or erythrosin orange G as a counterstain.

PREVIOUS RECORDS OF HERMAPHRODITISM

Previous records of hermaphroditism in limpets are limited to *Patella vulgata*. Gemmill (1896) examined approximately 250 specimens of *P. vulgata* and obtained three hermaphrodite gonads. They were normal in size, shape and anatomical position, but their colour was mottled 'showing all shades between olive-green of ovary and the light yellow of testis'. All regions appeared fully ripe, and Gemmill stated that microscopic examination showed 'not only ripe ova and spermatozoa but also segmented ova and even ciliated freely moving embryos'. One specimen showed a patchy distribution of male and female tissue with an excess of ovarian over testicular tissue; in the second specimen, one side of the gonad was purely male, whereas the other side was female, and in the third specimen only a small patch of ovarian tissue was found. Gemmill's remarkable record of developing ova and trochophores in these gonads is quite unique: in spite of a careful search during the present work no developing eggs have been found in the gonads examined. Pelseneer (1926) found one hermaphrodite gonad in 2750 specimens examined: it came from an individual 53 mm in length and in describing it he says 'c'était un testicule dans lequel, entre les acini mâles il y avait des rangées rectilignes d'ovules'. Orton (1928) records that in his investigations at Plymouth he never encountered hermaphroditism of the type described by Pelseneer. M. D. Jones (1933, unpublished record) examined 3000 specimens of *P. vulgata* and found only one hermaphrodite. This specimen was 46 mm in length and the gonad contained mainly testicular tissue: there was a small ovarian region at the anterior end. The two areas were fairly distinct apart from a few ova deeply embedded in testicular tissue. Smith (1935) examined more than 1000 specimens of *P. vulgata* and found only one hermaphrodite. Relevant data concerning these previous records are summarized in Table I.

STRUCTURE OF THE GONADS

The structure and development of the normal limpet gonad have been described and illustrated in a previous paper (Orton, Southward & Dodd, 1956). In connexion with this work many gonads of both sexes at all stages of

TABLE I. *PATELLA VULGATA:* PREVIOUS RECORDS OF HERMAPHRODITISM

Author	Date	Locality	No. of specimens examined	No. of herm-aphrodites	% herm-aphroditism
Gemmill	1896	Millport	*c.* 250	3	*c.* 1·2
Pelseneer	1926	Wimereux	2750	1	0·036
Orton	1928	Plymouth	1000+	0	Nil
Jones*	1933	Aberystwyth	3000	1	0·033
Smith	1935	Plymouth	1000+	1	<0·1

* Unpublished record.

development and depletion were sectioned and examined, and all were entirely of one sex. In both sexes the gonad lies on the ventral surface of the visceral mass and, initially, the germinal epithelium lines a flattened sac-like structure. This epithelium later becomes greatly folded and the folds, supported on trabeculae of connective tissue, penetrate the cavity and obliterate it. Gametogenesis appears to be further advanced in the dorsal regions of the gonad, i.e. those in contact with the visceral mass.

The *hermaphrodite* gonads are individually described in Table III (see pp. 335–8), together with the dates of collection, length of foot or shell and maximum thickness of gonad: these data are of significance when considering the relationship between the types of hermaphroditism here described and the transient hermaphroditism which may be associated with sex change.

Reference to Pl. I shows that, with the exception of two specimens containing trematode parasites, the hermaphrodite gonads showed well-defined areas of testicular and ovarian tissue, with some slight intermingling in the contiguous zones. Pl. I, fig. 6, illustrates a feature which was relatively frequent, viz. the presence of well developed oocytes lying in the lumen of a seminiferous tubule. In most cases these oocytes were continuous with more extensive ovarian tissue, but in a few cases they were isolated and completely surrounded by testis.

Pl. I, fig. 7, shows details of a typical region lying between predominantly male and predominantly female areas. Fully developed eggs and sperms are present in close proximity, together with young oocytes and a considerable amount of undifferentiated tissue.

Most of the gonads studied showed well developed oocytes and spermatozoa. In a few cases there were undoubted signs of spawning from both ovarian and testicular regions. From one hermaphrodite gonad a successful artificial fertilization was made between eggs and sperms, and apparently normal trochophores were obtained (cf. Dodd, 1956).

The relative volumes of ovarian and testicular tissue in each individual hermaphrodite gonad were estimated approximately. Of the twenty-six gonads available for histological study, fifteen were predominantly male, nine predominantly female, and in two of them the two types of tissue were of approximately equal volume.

INCIDENCE OF HERMAPHRODITISM

When considering the incidence of hermaphroditism encountered in the present study it is important to note that Orton's examination of the gonad was limited to size and general appearance of the ventral surface. Orton was mainly interested in establishing details of the breeding cycle. As he recognized, such an examination might well result in failure to identify hermaphrodite gonads in which the ventral surface was largely or entirely of one sex, e.g. specimen B 13, Table III. However, the 5844 specimens of *P. aspera* from the Isle of May, studied by the present author, were specifically examined for signs of hermaphroditism, and it is unlikely that any cases were overlooked. It is significant that the percentage frequency of hermaphroditism in this population is closely similar to the average percentage frequency for all localities.

TABLE II. HERMAPHRODITISM IN BRITISH SPECIES OF *PATELLA*: INCIDENCE AT DIFFERENT LOCALITIES

Locality	No. examined	No. of hermaphrodites found	Percentage hermaphroditism
	Patella vulgata		
Aberdeen	4,204	Nil	Nil
St Andrews	7,744	2	0·026
Isle of May	1,507	1	0·066
Millport	4,451	1	0·022
Cullercoats	4,583	Nil	Nil
Port St Mary	8,777	1	0·011
Plymouth	6,450	Nil	Nil
Trevone	5,541	Nil	Nil
All above localities	43,257	5	0·012
	Patella aspera		
Aberdeen	1,436	2	0·139
Isle of May	5,844	7	0·120
Port Erin	688	2	0·291
Port St Mary	3,633	5	0·138
Plymouth	2,125	6	0·282
Trevone	2,761	2	0·072
All above localities	16,487	24	0·146
	Patella depressa		
Gwbert	338	Nil	—
Plymouth	40	Nil	—
Trevone	4,454	1	0·022
All above localities	4,832	1	0·021

The overall percentage frequencies of hermaphroditism in the three species show interesting differences. Of 43,257 specimens of *P. vulgata* examined five were hermaphrodite: a percentage of 0·012; of 16,487 specimens of *P. aspera*, twenty-four were hermaphrodite: a percentage of 0·146; while in *P. depressa* only one hermaphrodite was found in 4832 specimens. It therefore appears that hermaphroditism is some ten times more common in *P. aspera* than in *P. vulgata* or *P. depressa*.

By reference to Table III it will be observed that the majority of the hermaphrodite specimens were taken during the spawning season of the particular species. This simply reflects the fact that by far the majority of the individuals examined were collected during the breeding season. It will be realized that hermaphrodite gonads can be recognized by superficial examination only when they are active.

The records suggest that hermaphroditism in *P. vulgata* may be more common in more northern localities: this, however, does not appear to be so in *P. aspera*.

Hermaphroditism in Relation to Sex Change

As has already been mentioned, a change of sex appears to take place in *P. vulgata* and *P. coerulea*. Since incomplete sex-change would necessarily result in hermaphroditism it is important to consider to what extent this phenomenon might account for the hermaphrodite gonads encountered in the present study.

Bacci (1947) has made a detailed investigation of sex-change in *P. coerulea*. In this species it appears that sex inversion is not limited to a narrow size range; very immature oocytes appear in male gonads, though this is true only of gonads which are almost completely spent: and gonads are never found in which there are well-defined male and female zones. Clearly then, in *P. coerulea*, although more than 15,000 specimens were examined by Bacci no hermaphrodites of the kind described in this paper were ever encountered.

Regarding *P. vulgata*, Orton (1928) has shown that most, if not all, of the individuals are male at their first sexual maturity, the suspected change of sex from male to female occurring at an age of 1 year or more, though more frequently within the smaller size-groups. The percentage frequencies of the two sexes approach equality in the second year-group. In the present study of some 43,000 specimens of *P. vulgata*, only 5 (0·012%) hermaphrodites were found, and most of these belonged to the larger size-groups.

As regards the remaining British species, Dr A. J. Southward is at present preparing for publication some of Orton's later data on sex-change, and he informs me that these indicate a different state of affairs. In *P. aspera*, if indeed sex-change occurs at all, it is a much rarer phenomenon since 30–40% females are already present in the smallest size-groups in which all individuals show developing gonads. In *P. depressa*, there is strong circumstantial evidence that no sex-change ever occurs, 70% males being found in the size-groups in which all specimens have differentiated gonads. Evidently, therefore, sex-change is not an essential prerequisite for hermaphroditism, though in *P. vulgata* and *P. aspera* a possible connexion between the two phenomena must be borne in mind.

Hermaphroditism in Relation to Parasitic Infestation

Although the hermaphrodite condition is itself abnormal, both male and female parts of hermaphrodite gonads were of normal histological appearance in most individuals. Moreover, evidence that functional spermatozoa and ova may both be produced within a single gonad has already been adduced. In six of the specimens examined, however, the gonads were recognizably abnormal not only through being hermaphrodite, but also in gross morphology (Pl. I, fig. 2). In three of these there was no evidence as to the cause of the abnormality, whereas in the remaining three, the possibility must be considered that parasitic infestation was responsible. In two of the three parasitized specimens rediae of *Cercaria patellae* Lebour were found (Pl. I, fig. 8), whilst in the other several cysts of the cestode *Ophryocotyle* sp. were present (Pl. I, fig. 9). Rees (1934) has described the effects produced by *Cercaria patellae*, and her main observations concerning partial castration are in full agreement with my own. Rees, however, did not encounter hermaphroditism. The parasitized hermaphrodite gonads which I have studied were subnormal in size and contained both ova and spermatozoa indiscriminately mixed. Many of the ova showed gross degeneration, and large areas of the gonad were entirely devoid of germ cells. It should be noted that a high proportion of most populations of limpets may be parasitized by *C. patellae* and since only two parasitized hermaphrodite specimens were found in the present study the possibility must be borne in mind that these specimens were already hermaphrodite when infested by the parasite, i.e. that they were not rendered hermaphrodite by the parasite.

The parasitized specimen (Pl. I, fig. 9) containing cestode cysts closely resembled the unparasitized hermaphrodites. The gonad was predominantly female and the ova were mostly large. A well-developed male area and some neuter tissue were associated with the largest cyst. There were restricted areas of neuter gonad tissue surrounding most of the other cysts: in one case the adjacent tissue, though predominantly neuter, contained early stages in spermatogenesis. However, since there were male regions not associated with the presence of cysts, this gonad also may possibly have already been hermaphrodite before infestation by the parasite.

DISCUSSION

In order to assess the relationship between the type of hermaphroditism here described and the unisexual condition normal in limpets, two hypotheses may be considered. According to one hypothesis, since in *Patella vulgata* sex reversal apparently occurs in the great majority of individuals, hermaphrodite gonads may represent an arrest of developmental change during sex-reversal. Consideration has already been given to this possibility and it would appear that sex-change does not predispose the gonad towards hermaphroditism since the incidence of hermaphroditism in all three British species is low, whereas sex-change in *P. vulgata* must occur in at least 90% of individuals. It has also been noted that the gonads here described may contain fully developed eggs and sperms at one and the same time: Bacci (1947) found that this was never so in *P. coerulea*, in which he found the sexual phases to be relatively stable, sex-change apparently occurring during the resting period. Moreover, in nearly all of the cases here described, the hermaphrodite gonads were found in large individuals, i.e. those past the time at which sex-change is most commonly thought to occur.

According to a second hypothesis, since sexuality is probably labile in limpets the small percentage of hermaphroditism may well merely represent aberrant sexuality with either a genetical or an environmental basis. In this connexion, Coe (1945) states that although less than 400 of the more than 10,000 species of lamellibranchs are normally hermaphrodite, in most of the unisexual species which have been studied a small percentage of hermaphrodite individuals has been found. He states: 'Certain dominantly unisexual species may have local races or environmental modifications which are hermaphroditic. Furthermore, accidental or developmental hermaphroditism is of occasional occurrence in most of the unisexual species and unisexual individuals are sometimes found in dominantly hermaphroditic species.' A consideration of all the evidence in the present study leads irresistibly to the conclusion that we are here dealing with a similar form of accidental hermaphroditism for which no definite cause can be assigned at present. The low incidence appears to rule out the possibility that it is a factor associated with the external environment, though it has been shown that an abnormal internal environment due to the presence of cestode cysts can result in a fairly clear local action on the gonad structure. So far as concerns a possible genetical explanation of this phenomenon, Montalenti (1950) and Montalenti & Bacci (1951) have put forward a hypothesis that sex in *Patella* is determined by multiple genes. They postulate three pairs of alleles which segregate independently and can therefore produce sixty-four possible genotypes, one of which is a genetic male and one a genetic female, these two genotypes being represented by the small percentage of individuals which do not change sex. The remaining genotypes contain both male and female potentialities and any individual possessing one of these genotypes could, presumably, develop into a hermaphrodite. It should be noted that this genetical scheme was suggested to cover the entire genus, when it was thought that all species of *Patella* showed sex-change. This scheme may be considered to have less validity now that it appears that several of the species are truly gonochoristic. No doubt a formal genetical scheme could be arrived at which would cover all the known facts regarding sexuality in the genus *Patella* and allow for occasional hermaphroditism, though it would seem unlikely that any such scheme could account for the peculiar hermaphroditism here described. Conceivably these *Patella* hermaphrodites are developmental mosaics in which islands of germinal tissue originate from cells which by rare mitotic accident happen to be heteroploid. There is no case for elaborating this statement in view of the present lack of knowledge of molluscan chromosomes.

Summary

In an examination of 64,576 specimens of the three British species of *Patella*, thirty hermaphrodites were encountered. The present paper describes the gonads of these animals, and discusses and compares the incidence of hermaphroditism in limpets from widely separated British localities. Three parasitized hermaphrodite gonads are described, and it is suggested that these were probably already hermaphrodite at the time of infestation by the parasite.

Consideration is given to the possibility that there is a causal connexion between sex-change and hermaphroditism, but such a connexion appears not to be supported by the available evidence.

The nature of this hermaphroditism is considered, and it is concluded that we are here dealing with accidental or developmental hermaphroditism for which no cause can be assigned, though it is tentatively suggested that the gonads may be developmental mosaics containing islands of heteroploid tissue.

TABLE III. HERMAPHRODITE LIMPET GONADS: TABULATED DATA

Specimen	Date collected	Locality	Length of shell or foot (mm)	Maximum thickness of gonad (mm)	Description of gonad based on macroscopic appearance and examination of sections
			(1) *Patella vulgata*		
A 1	6. xi. 48	St Andrews	41·0 (shell)	7·0	Predominantly female. Great intermixing of the two elements though essentially a skin of testis over eggs which come to surface in centre region. Eggs and sperms appear mature, though there are a fair number of young eggs
A 2	5. iii. 49	St Andrews	27·0 (foot)	3·0	Male and female areas approximately equal in volume and greatly intermixed. Connective tissue septa with young oocytes have penetrated deeply into testis tissue
A 3	16. x. 48	Isle of May	15·0 (foot)	2·0	Predominantly female, only one small male area. Abnormal distribution of gonad—intermixed with gut. Eggs and sperms well developed, very few small eggs
A 4	29. x. 48	Millport	70·0 (shell)	8·0	Predominantly female. Very abnormal with irregular lobed appearance. Ovarian region forms a large excrescence. Two regions quite distinct except at edges where there is some over-growing of female elements by male elements
A 5 (Pl. I, fig. 6)	23. iii. 46	Port St Mary	30·0 (foot)	2·0	Predominantly male. Small discrete patches of eggs in centre region which extend to visceral mass. Large oocytes found in testicular tissue

TABLE III (*continued*)

Specimen	Date collected	Locality	Length of shell or foot (mm)	Maximum thickness of gonad (mm)	Description of gonad based on macroscopic appearance and examination of sections
				(2) *Patella aspera*	
B 1	31. x. 46	Aberdeen	—	2·0	Predominantly female. Both medium-sized and small eggs well represented. Male and female regions greatly intermixed. Testis tissue appears spent and contains many young oocytes; ovarian tissue seems to be developing
B 2 (Pl. I, figs. 5, 7)	21. x. 47	Aberdeen	25·0 (foot)	3·0	Predominantly male. Testis region consists of a large bilobed mass. A lobe of ovarian tissue lies at the anterior end, a wedge-like extension of which overlies the adjacent testis region. Most eggs seem fully developed though there are some early developmental stages at the ventral surface. Testis region contains fully developed spermatozoa and all other stages in spermatogenesis are well represented
B 3	23. viii. 48	Isle of May	50·0 (shell)	6·0	Male and female areas approximately equal in extent. Two well-demarcated areas: testis overlies ovary. Eggs and sperms well developed though developmental stages are present, especially in testicular region. A few isolated large eggs are seen deep in testis tissue. Signs of spawning from ovarian tissue only
B 4	6. ix. 48	Isle of May	31·0 (foot)	5·0	Predominantly male. Fairly discrete crescent-shaped area of ovarian tissue which extends throughout thickness of gonad—little intermixing of the two areas. Both contain apparently fully developed genital products as well as developmental stages. Signs of spawning
B 5	6. ix. 48	Isle of May	31·0 (foot)	9·0	Predominantly male. Two large patches of testis separated by ovarian tissue containing several small islands of testicular tissue. Great intermixing in these regions. All areas contain fully developed genital products as well as developmental stages
B 6 (Pl. I, fig. 1)	7. ix. 48	Isle of May	29·0 (foot)	8·0	Predominantly male. Consists of two large areas of testis separated by a wedge of ovarian tissue running across the shorter axis of the gonad. Some intermixing. Genital products mostly apparently fully developed. Some signs of spawning in both areas
B 7	19. ix. 48	Isle of May	50·0 (shell)	2·5	Predominantly female. Consists of a layer of ovarian tissue covering the visceral mass, with fairly discrete areas of testicular tissue embedded in the more ventral regions. Female area consists mainly of large eggs: some eggs may have been spawned. Much mature spermatozoa and all stages in spermatogenesis. Patches of sperms on connective tissue septa deep in ovarian regions

B 8	21. ix. 48	Isle of May	55·0 (shell)	4·0	Predominantly male with patches of ovarian tissue which are fairly discrete. Female areas appear more mature than male areas. No sign of spawning
B 9	23. ix. 48	Isle of May	51·0 (shell)	7·0	Predominantly (*c.* 90 %) male. Dorsal region consists of a loose mass of tissue with a few scattered large eggs and a small amount of relict sperm. Ventral male area very well developed. Much fully developed sperm and many earlier stages in spermatogenesis. Dorsal regions of gonad appear spent (both male and female areas)
B 10	5. vi. 47	Port Erin	35·0 (foot)	1·5	Predominantly male with a few areas of fairly discrete ovarian tissue. Little intermixing. Testis appears almost spent. Ovary consists of a few medium-sized eggs and many small oocytes—may be an early developmental stage
B 11	25. vi. 48	Port Erin	—	3·0	Predominantly male. Great intermixing of ovary and testis, though there is fairly distinct demarcation between them in the regions where they adjoin. Ovary and testis extend from surface to base of gonad and both appear fully developed; some spawning seems to have taken place from both ovary and testis
B 12 (Pl. I, fig. 2)	9. xii. 46	Port St Mary	60·0 (shell)	—	Not available for histological examination. Gonad obviously very abnormal and predominantly male
B 13	12. ix. 47	Port St Mary	—	3·0	Predominantly male. A continuous skin of male tissue overlying basal eggs. Testis appears to be developing; eggs seem mostly mature. Very little intermixing. Eggs visible on macroscopic examination of gonad only at the edges
B 14 (Pl. I, fig. 4)	4. x. 47	Port St Mary	27·0 (foot)	4·0	Predominantly male. Consists of many scattered islands of ovarian tissue lying on ventral surface of a mass of testis tissue. These islands are fairly discrete and some penetrate entire thickness of gonad and reach visceral mass. Both eggs and sperms seem fully developed and there appears to have been some spawning
B 15	15. ix. 48	Port St Mary	48·0 (shell)	2·5	Predominantly male. Fairly discrete small islands of ovarian tissue consisting of large eggs are scattered throughout the gonad. A few rediae of *Cercaria patellae* are present: testis tissue in which they lie looks quite normal. Male areas less well developed than female areas
B 16	28. i. 49	Port St Mary	30·0 (foot)	0·05	Predominantly male with female area at anterior end. Both regions contain fully developed genital products, though both are almost spent. A few large eggs can be seen along dorsal margin of gonad. Young oocytes are found in fair numbers in the testis tissue

TABLE III (*continued*)

Specimen	Date collected	Locality	Length of shell or foot (mm)	Maximum thickness of gonad (mm)	Description of gonad based on macroscopic appearance and examination of sections
B 17 (Pl. 1, fig. 9)	17. vi. 47	Plymouth	39·0 (foot)	1·6	Predominantly female. Very abnormal in microscopic appearance. Heavily parasitized with rediae of *Cercaria patellae*. Some large eggs and apparently fully developed sperms are present in isolated patches and are completely intermixed. Some eggs appear to be degenerating
B 18 (Pl. I, fig. 8)	30. x. 47	Plymouth	30·0 (foot)	6·0	Predominantly female with a skin of testis tissue over most of ventral surface. Eggs and sperms seem fully developed and there has been some spawning. A large cyst of *Ophryocotyle* sp. is present near centre of ventral region of gonad and in this area the greatest intermixing occurs. Although it lies mainly in the ovarian tissue, there is a large cap of testis tissue covering its ventral surface
B 19	3. xi. 47	Plymouth	—	—	Not available for histological examination
B 20	8. v. 49	Plymouth	50·0 (shell)	2·5	Predominantly male. Patches of large eggs present, many of which inter-communicate along dorsal surface of gonad. Both testicular and ovarian regions appear mature. Dorsal region of testicular area almost spent
B 21 (Pl. I, fig. 3)	3. iii. 49	Plymouth	35·0 (foot)	4·0	Predominantly female. Large island of testicular tissue in central region of gonad. This lies in a hollow in the ovarian tissue and does not penetrate as far as dorsal surface of gonad. Great intermixing of male and female tissues at edges. Testicular tissue penetrates deeply into surrounding ovarian region. Few small oocytes
B 22	28. vi. 49	Plymouth	—	—	Not available for histological examination
B 23	19. viii. 47	Trevone	—	—	Not available for histological examination
B 24	2. ix. 47	Trevone	—	4·0	Predominantly female. Eggs and sperms appear mature and show some intermixing
			(3) *Patella depressa*		
C 1	2. ix. 48	Trevone	24·0 (foot)	6·0	Predominantly male. Ovarian tissue forms a thin skin on the ventral surface—does not penetrate very deeply into thickness of gonad. Eggs large in size, look somewhat abnormal and are separated by a network of tissue in which spermatogenesis is proceeding—a few fully formed sperms. Male areas less mature than female areas

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EXPLANATION OF PLATE I

P. aspera and *P. vulgata*: morphology and histology of hermaphrodite gonads.

Fig. 1. *P. aspera*; specimen B6. Approx. natural size. Gonad and visceral mass freed from foot and suspended from it to show ventral surface of gonad. Testicular areas white, ovarian areas black.

Fig. 2. *P. aspera*; specimen B12. Approx. natural size. Gonad and visceral mass freed from foot and the latter deflected forward to show ventral surface of gonad. Testicular areas white, ovarian areas black. Note abnormal appearance of gonad.

Fig. 3. *P. aspera*; specimen B21. Approx. natural size. Arrangement of parts similar to fig. 1. Note discrete island of testicular tissue.

Figs. 4–9 inclusive. T.S. hermaphrodite gonads. Plane of section parallel to dorso-ventral axis.

Fig. 4. *P. aspera*; specimen B14. × 10.

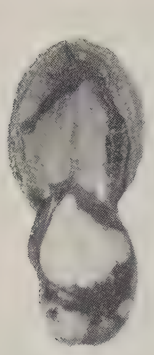
Fig. 5. *P. aspera*; specimen B2. × 10.

Fig. 6. *P. vulgata*; specimen A5. × 16. Note large oocytes in seminiferous tubule.

Fig. 7. *P. aspera*; specimen B2. × 125. High-power view of region between male and female areas; see fig. 5. Note part of large oocyte at lower edge, isolated groups of spermatozoa, young oocytes and neuter tissue.

Fig. 8. *P. aspera*; specimen B18. × 25. Hermaphroditism associated with parasitization. Note that tissue in immediate neighbourhood of cyst of *Ophryocotyle* is either testicular or neuter.

Fig. 9. *P. aspera*; specimen B17. × 32. Hermaphroditism associated with parasitization. Note grossly abnormal appearance of gonad and presence of isolated oocytes, patches of testicular tissue and rediae of *Cercaria patellae*.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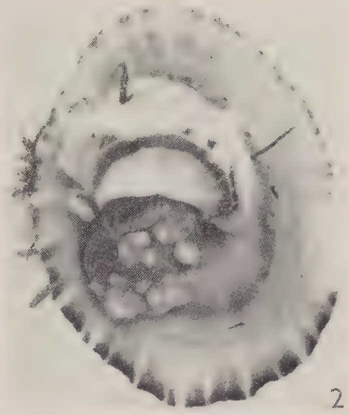
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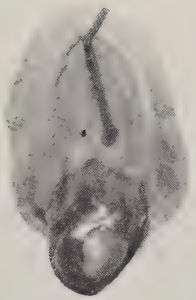
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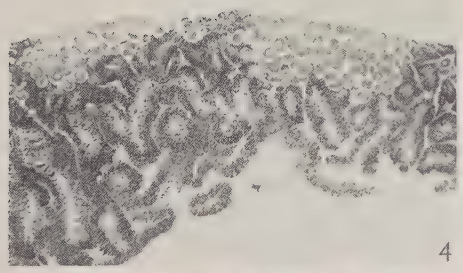
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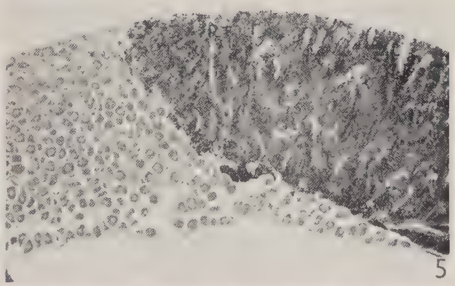
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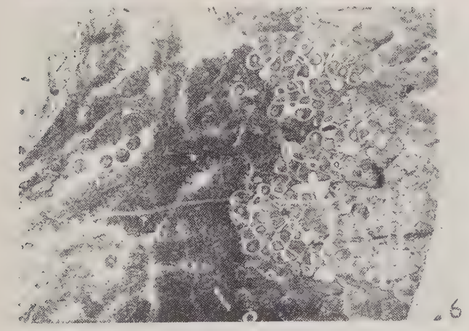
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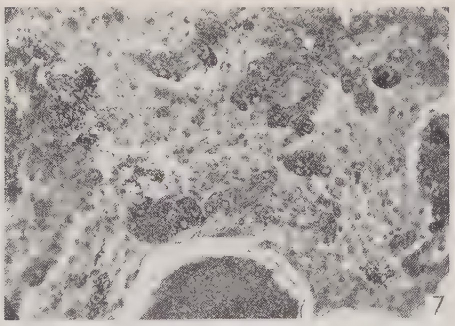
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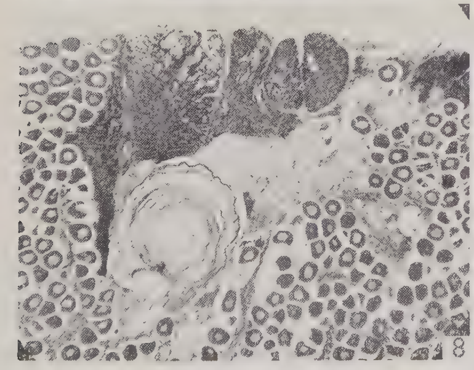
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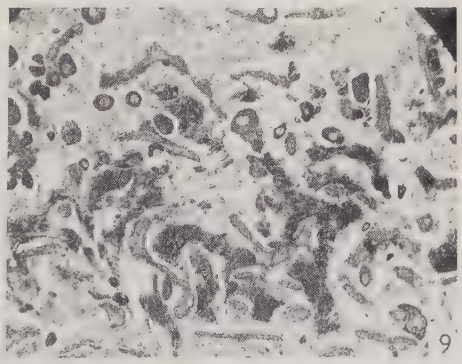
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ON ASSESSING THE AGE OF DEEP OCEANIC WATER BY CARBON-14

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(Text-fig. 1)

The rate of circulation and age of the deep water of the oceans is of much interest. Worthington (1955) has suggested an age of 100–160 years for northern North Atlantic water which has reached the Carribean and Cayman Seas. I (in part, Cooper, 1955, 1956) have suspected that the rate of circulation of much of the North Atlantic deep water may be even faster than Worthington's results suggest. Provisional direct observations by G. Wüst & G. Dietrich (private communication) also suggest that the deep circulation is quite rapid.

In strong contrast, Kulp (1952, 1953*a*, *b*) and Carr & Kulp (1954) have attributed ages of 1600 to 1950 years to seawater samples drawn from various depths in the North Atlantic (Table I, nos. 6–9*a*).

The discrepancy is not real, but arises from tacit assumptions as to the nature and age of a water mass which are incompatible and from peculiarities of the cycle of $^{12}CO_2$ and $^{14}CO_2$ in nature.

Kulp (1952) wrote: 'Although atmospheric carbon dioxide is in equilibrium with the carbonate in surface ocean water, the submergence of such water cuts it off from its supply of carbon 14 as effectively as does death in the case of a plant or animal.' The first clause requires proof by observation. It would be better to replace 'is in equilibrium' by 'approaches equilibrium'.

He went on: 'Since ocean water sinks in the polar regions and moves along the bottom towards the equator, the rate and direction of movement can be measured by determining the time since a unit of water left the polar surface.'...'The carbon 14 measurements of several water samples taken from the ocean floor at about the latitude of Newfoundland on either side of the Mid-Atlantic Ridge are shown in Table 4' (included here as Table I, nos. 5–9*a*). 'The data suggest that it takes about 1500 years for the water to reach this latitude from the Arctic. Thus the time of the turnover of the oceans must be thought of in terms of several thousand years.'

In a way the whole of this statement is true; but it is a way of no value to an oceanographer seeking a better understanding of oceanic circulation. This approach may be compared with that of a man who would measure the strength of currents in the North Sea with drift bottles, not knowing that between putting out the bottles and their stranding on a shore they had many times circled the northern North Sea Great Eddy (Tait, 1937). His results

TABLE I. AGE OF OCEANIC WATER ACCORDING TO J. L. KULP

Reference Kulp	Location	Sea area	Depth*		Apparent age (years)
			ft.	m.	
1. 1953 *a*	13° 35′ N., 66° 35′ W.	Caribbean	Surface	—	Recent
2. 1953 *a*	19° 24′ N., 78° 33′ W.	South of Cuba	Surface	—	Recent
3. 1953 *a*	41° 00′ N., 54° 35′ W.	West North Atlantic, south of Newfoundland	Surface	—	Recent
3 *a*. 1953 *b*	54° 35′ N., 41° W.	West North Atlantic	Surface	—	Recent
4. 1953 *a*	63° 46′ N., 00° 26′ W.	Norwegian Sea	10,440	*3182*	500 ± 200
5. 1953 *a*	38° 42′ N., 67° 54′ W.	North-west North Atlantic	13,500	*4115*	450 ± 150
6. 1953 *a*	35° 46′ N., 69° 05′ W.	North-west North Atlantic	15,300	4663	1950 ± 200
7. 1953 *a*	34° 56′ N., 68° 14′ W.	North-west North Atlantic	16,560	*5047*	1550 ± 300
8. 1953 *a*, 1953 *b*	58° 19′ N., 32° 57′ W.	Reykjanes Ridge, Western Slope	6,000	1829	1600 ± 130
9. 1953 *b*	53° 53′ N., 21° 06′ W.	Southern tip of Rockall Bank	*9,000*	2743	1750 ± 150
9 *a*. 1953 *a*	53° 53′ N., 21° 06′ W.	Southern tip of Rockall Bank	9,100	*2774*	1900 ± 150

* Roman figures give depths reported by Kulp: italic figures conversions by the writer.

would suggest a much more sluggish circulation than would measurements made with current meters.

To appreciate what is involved, a number of separate points in the physical chemistry and oceanography of carbon dioxide have to be examined and then synthesized into a complete whole. The material for such a study has been provided by Buch (1942, *inter alia*).

A COMPARISON BETWEEN THE RATES OF EXCHANGE OF O_2, $^{12}CO_2$ AND $^{14}CO_2$ BETWEEN AIR AND SEA

Let P_0, V_0 = the partial pressure and volume of a gas in the surface water of the sea when in equilibrium with the atmosphere under specified conditions.

P, V = the partial pressure and volume of a gas when not in equilibrium.

γ = an exchange coefficient, assumed to be the same for all three gases.

t = time.

Then
$$\frac{P}{P_0}=\frac{V}{V_0}.$$

Also, the rate of invasion or evasion of a gas from the atmosphere into the sea, or vice versa, may be written

$$\frac{\mathrm{d}V}{\mathrm{d}t}=\gamma(P_0-P)=\frac{\gamma P_0}{V_0}(V_0-V),$$

or
$$\mathrm{d}t=\frac{V_0}{\gamma P_0}\,\frac{\mathrm{d}V}{V_0-V}.$$

On integration
$$\mathrm{t}=\int_{V=V_1}^{V=V_2}\mathrm{d}t=\frac{V_0}{\gamma P_0}\log_e\frac{V_0-V_1}{V_0-V_2}.$$

Let us now consider a litre of water from which $2x$ ml. of O_2 and $2x$ ml. of $^{12}CO_2$ have been removed, and then compute the ratio of the times required to reabsorb x ml. of each gas. For both gases:

$$\log_e \frac{V_0 - V_1}{V_0 - V_2} = \log_e \frac{2x}{x} = \log_e 2.$$

Consequently

$$\frac{t_{\text{carbon-12 dioxide}}}{t_{\text{oxygen}}} = \frac{V_{0,\ \text{carbon-12 dioxide}}}{V_{0,\ \text{oxygen}}} \cdot \frac{P_{0,\ \text{oxygen}}}{P_{0,\ \text{carbon-12 dioxide}}}.$$

Let us now compute this ratio (Table II) for moist air conditions in the Norwegian Sea and in the tropical Atlantic, using the tables of Truesdale, Downing & Lowden (1955) for oxygen, and of Buch (1933) for carbon dioxide.

TABLE II. RELATIVE TIMES REQUIRED BY CARBON-12 DIOXIDE AND BY OXYGEN TO APPROACH EQUILIBRIUM WITH THE ATMOSPHERE WHEN UNDERSATURATED OR SUPERSATURATED TO THE SAME EXTENT

	Norwegian Sea	Tropical Atlantic
Temperature (° C)	0	25
Salinity (‰)	34·9	36·5
V_0, $\Sigma\,^{12}CO_2$ (ml./l.)	49·5	47·5
V_0, O_2 (ml./l.)	8·08	4·68
P_0 O_2 (atm.)	0·209	0·203
P_0, $^{12}CO_2$ (atm.)	0·00033	0·00033
Ratio of times, $t_{^{12}CO_2}/t_{O_2}$	*3880*	*6244*

Thus, when $^{12}CO_2$ and O_2 are displaced from their equilibrium values by the same amount, then $^{12}CO_2$ requires between 4000 and 6000 days (between 10 and 17 years) to achieve an approach towards an equilibrium which oxygen may attain in 1 day.

This is a general conclusion, independent of the storminess of the sea and the actual rates of transfer. It is equally true whichever side of equilibrium the departure may be made. Whenever oxygen is not in equilibrium, it is highly unlikely that carbon-12 dioxide will be so.

When $^{14}CO_2$ is at an overall equilibrium state in air and water, its partial pressure and its volume will both bear the same ratio to the partial pressure and volume of $^{12}CO_2$. The ratio V_0/P_0 will be essentially the same for both isotopes so that the above calculation applies.

In general, however, due to radio-active decay, the volume and partial pressure of $^{14}CO_2$ in the water will tend to diminish. Consequently, $^{14}CO_2$ may require even longer to approach towards equilibrium than does $^{12}CO_2$.

ON THE CONCENTRATION GRADIENT OF CARBON DIOXIDE BETWEEN THE EQUATOR AND THE POLES

Kulp (1953*a*) has said that 'the C^{14} concentration in air at widely different geographic positions is essentially constant and is independent of time of day, rainfall, altitude and temperature'.

This apparently straightforward statement may mean either (*a*) the concentration of ^{14}C per litre of air at N.T.P. is constant; or (*b*) that the ^{14}C concentration per unit volume of carbon dioxide ($^{12}CO_2 + ^{13}CO_2 + ^{14}CO_2$) is constant.

The alternatives are not closely related. Interpretation (*a*) is probably the one intended. However, in the same paragraph he stated that 'about two dozen living trees from all over the world gave the same carbon-14 concentration within about 10%'. This implies that the carbon-14 content is proportional to the amount of carbon dioxide assimilated and supports interpretation (*b*).

The atmospheric circulation over the North Atlantic according to Bjerknes and the distribution of carbon dioxide ($^{12}CO_2$) according to Buch are shown schematically in Fig. 1 (Buch, 1942).

In temperate latitudes the partial pressure of carbon dioxide in the air and in surface water in equilibrium with the air is usually about $3 \cdot 1 \times 10^{-4}$ atm. In summer, in extensive areas of the Arctic, this partial pressure may sink as low as $1 \cdot 5 \times 10^{-4}$ atm. Polar regions in summer are the site of much solution in the sea of atmospheric carbon dioxide both as $^{12}CO_2$ and $^{14}CO_2$. There are no winter observations in the Arctic.

In the Antarctic south of 57° S. lat. in winter (Deacon, 1940) the partial pressure in surface water is about $3 \cdot 3 \times 10^{-4}$ atm. If this figure applies in the central Norwegian Sea where deep water is formed, we may reasonably assume that in winter the water sinks with the properties shown in Table III.

If a parcel of this water, sealed against gain or loss of everything except heat, were transported to the tropics and warmed to 25° C, the equilibria of the carbonate system would be strongly displaced. The partial pressure and pH would acquire the values also shown in Table III, calculated from Buch (1933). The increase in partial pressure of CO_2 is a purely physico-chemical effect. Living organisms and solution or deposition of calcium carbonate have nothing to do with it. Failure to appreciate this effect of temperature change has led to much loose thinking about decay processes in the sea. Since partial pressure of CO_2 and pH may be so misleading, only from the total concentration of CO_2 may sound conclusions be drawn about regeneration processes.

This is essentially a thermodynamic argument, so that the history of the parcel of water between sinking near the poles and upwelling by some means in the tropics is immaterial. The conclusion is that, in an azoic world, upwelling water in the tropics must have a partial pressure of $^{12}CO_2$, greatly in excess of the 'equilibrium value' in the tropical atmosphere. In upwelling areas the overlying air must be locally enriched. To balance this circulation of $^{12}CO_2$ in the oceans between poles and tropics a considerable concentration gradient of carbon dioxide is imposed upon the atmosphere between tropics and poles. This is what Buch found.

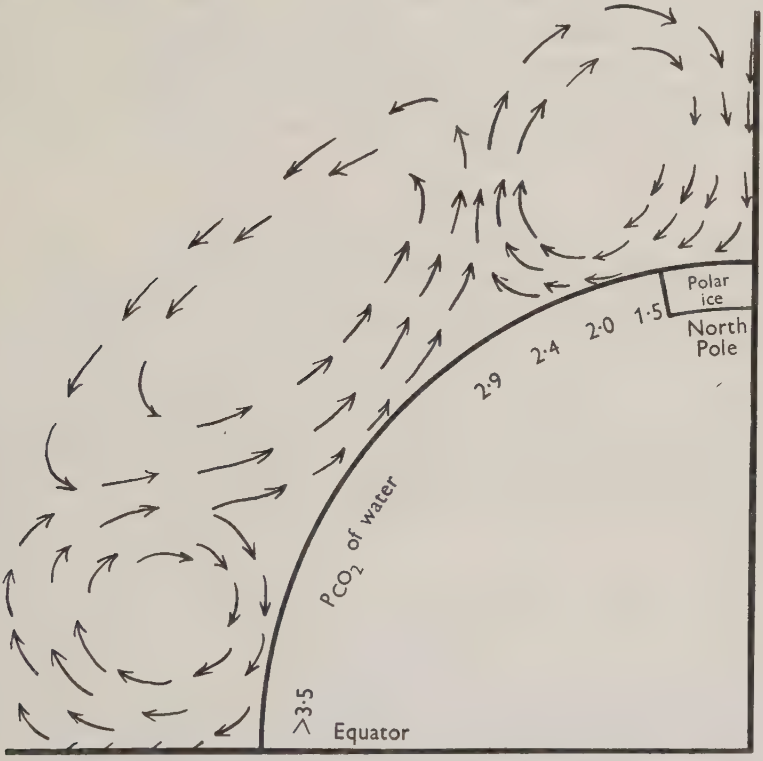

Fig. 1. Atmospheric circulation over the North Atlantic according to Bjerknes, and partial pressure of carbon-12 dioxide according to Buch. (After Buch, 1942.)

TABLE III

Temperature (° C)	−1 to −1·8	25
Salinity (‰)	34·92	34·92
Chlorinity (‰)	19·33	19·33
Partial pressure of CO_2 (atm.)	$3 \cdot 3 \times 10^{-4}$	$8 \cdot 6 \times 10^{-4}$
Σ CO_2 (m-mole/l.)	2·20	2·20
pH	8·10	7·84

From every point of view this upwelled water is 'old' so that the ratio $^{14}CO_2/^{12}CO_2$ must be low; so must the ratio in the gas which has there escaped to the atmosphere.

The equilibrium state between air and water is not static but dynamic, and means only that in unit time as many molecules of $^{12}CO_2$ or $^{14}CO_2$ enter the water as leave it. For the two isotopic molecular species, the balance may everywhere be struck differently. Often a tropical surface water may gain $^{14}CO_2$ from the air whilst losing $^{12}CO_2$.

If the air has arrived over an area of upwelling with an 'equilibrium' concentration of $^{14}CO_2$, referred either to the volume of air or to the volume of $^{12}CO_2$ in it, then addition of CO_2, poor in $^{14}CO_2$, must displace this equilibrium. It is clear that the total concentration of CO_2 in the air and the ratio of $^{14}CO_2$ to $^{12}CO_2$ should be subject to many changes in the course of a single cycle between tropics and poles and back. Either of the interpretations (*a*) and (*b*) on p. 344 may be true, but not both.

Isotopic Partition Functions

Partition functions of the reactions:

$$^{12}CO_2 + H^{14}CO_3' \rightleftharpoons {}^{14}CO_2 + H^{12}CO_3',$$

$$H^{12}CO_3' + {}^{14}CO_3'' \rightleftharpoons H^{14}CO_3' + {}^{12}CO_3'',$$

depend on temperature (Stranks & Harris, 1953). Between 0 and 28° the partition function of the first reaction changes from 0·957 to 0·965. At the higher temperature relatively more ^{14}C is free as $^{14}CO_2$ and can escape. The difference lies within present limits of error of the counting technique on carbon-14.

On Circulation and Recirculation of Carbon Dioxide in the Atlantic

Kulp's definition (quoted above) of the age of a water mass implies that there are bodies of water which make long journeys in the ocean whilst retaining all or nearly all their properties unchanged. No oceanographer who has studied the deep sea holds this view, but all are responsible in some measure for propagating it. The trouble starts when names such as 'North Atlantic deep water' or 'Antarctic bottom water' have to be devised to make complex concepts comprehensible. Because some dominant property may be traced for thousands of miles it is only too easy to assume that most of the water that accompanies the property has made the same journey in the same way at the same time. This is rarely so. There is no such thing as a pure-bred water mass. Attribution of a zero oceanographic age, therefore, implies some arbitrary selection of a time and place of birth and an understanding of subsequent mixing processes.

None the less 'water mass' is a useful and fruitful concept. Sometimes changes are so abrupt that it is possible to say that a new water mass has been born from well-defined parent water masses. More often exchanging and mixing processes are formless.

Thought is clarified by conceiving dominant characters of water masses. By a dominant character is meant one by which a water mass may be recognized after it has been mixed with a large proportion of other waters with poorer diagnostic characters. In this sense in the eastern North Atlantic, around 1000 m. depth, Mediterranean water is dominant after it has become mixed with many times its volume of North Atlantic Central water. The dominant characters are salinity, temperature-salinity relationship and the nitrate-phosphate ratio. In this case the characters of the North Atlantic Central water are all masked except for one—phosphate.

SINKING SOUTH-EAST AND SOUTH-WEST OF GREENLAND

This is the area where Helland-Hansen & Nansen (1909), Defant, Böhnecke & Wattenberg (1936), Smith, Soule & Mosby (1937), Wattenberg (1938) and Wust (1943) have considered that much deep water is formed. It is unquestionably an area where an enormous mass of water with markedly homogeneous properties is created. The mixing pot is at least 2000 m deep. Most oceanographers would consider this a good place to ascribe zero age to a water mass. If it is to have zero-age on the ^{14}C scale, the whole of this enormous volume of water would need to be completely equilibrated with the atmosphere for O_2, $^{12}CO_2$ and $^{14}CO_2$. An intensely vigorous process of exchange through the sea surface and of vertical circulation would be needed to achieve this. In fact it is not achieved, not even for oxygen.

Two of the most representative stations were Meteor stations nos. 121 and 122 (56° 37′ N., 44° 55′ W. and 55° 03′ N., 44° 46′ W.) worked on 9 March 1935 (Defant *et al.* 1936). Here, between 300 and 2000 m, oxygen lay between 90·8 and 96·2% saturated and averaged 93·0%.[1] Equilibrium with the atmosphere was not attained. It is certain that equilibrium could have been attained neither by $^{12}CO_2$ nor by $^{14}CO_2$. Consequently, if ^{14}C is to be a useful tool, its concentration in a newborn water mass such as this needs to be established empirically.

ON THE $^{14}CO_2$ CONTENT OF NORTH ATLANTIC DEEP WATER

Wattenberg (1938) and Wust (1943) have critically discussed the origin of the North Atlantic deep water and the Subarctic bottom current. They suggest that, from time to time, a pulse ('Einschübe') of Norwegian Sea water through the Denmark Strait may contribute to the deep water, but is not very important. The writer believes not only that such pulses are frequent, but that they dominate much of the oceanography of the North Atlantic. The evidence is being prepared for publication. Meanwhile, for the carbon-14 problem the following summary must suffice.

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[1] Recomputed from the oxygen saturation tables of Truesdale *et al.* (1955); the range is 92·1–98% and average 94·6%.

The North Atlantic deep water would seem to arise, not at any one place, but by a continuing process all the way from Jan Mayen to Labrador (Cooper, 1956). Many waters with varying contents of $^{14}CO_2$ will contribute to it.

Let us first consider the North Atlantic Drift and the Norwegian Sea.

The superficial waters of the North Atlantic Drift, subjected to intensive vertical mixing in winter but the site of intense photosynthesis in spring and summer, offer opportunity for equilibration of O_2, $^{12}CO_2$ and $^{14}CO_2$ between sea and atmosphere. Much of this water enters the Norwegian Sea and, with the drainage from North-western and Northern Europe, contributes to the surface water of the Norwegian Sea. This alone may be said to have zero age on both an oceanographic and a carbon-14 time scale. In old polar ice, which also contributes by melting, the ratio $^{14}CO_2/^{12}CO_2$ may be slightly low.

In the area of sinking of Norwegian sea water to form deep water the physical equilibrium becomes neutral so that equilibrium of O_2 and $^{12}CO_2$ between air and water might well be maintained to great depths; $^{14}CO_2$ is, however, constantly decaying. If the vertical mixing is so vigorous that all the water is equilibrated with $^{14}CO_2$ within a score or so of years then all of it may be said to have zero ^{14}C age. Since such vigour is unlikely, Norwegian Sea deep water at the time and place of sinking should have a positive ^{14}C age compared with North Atlantic drift surface water.

Over much of the polar basin there is an ice seal, complete in winter and broken by leads of brackish water in summer. Over the whole of the ice-covered seas exchange of gases between air and deeper Norwegian Sea water is prohibited. The ^{14}C age of this deeper water must be steadily increasing. Similarly, the cover of ice and brackish waters in the East Greenland Current provides a complete seal. Consequently, the water between 200 and 500 m depth in the Norwegian or Greenland Sea which is being sucked towards the passes into the Atlantic must have acquired a ^{14}C age measured in scores or, maybe, centuries of years.

This water undergoes mixing with other waters, few of which have been near the surface for a very long time. In the confined area of the Denmark Strait, the relatively warm and saline north-bound Irminger current is adjacent to the outgoing cold bottom current. It may contain some water which has equilibrated with the atmosphere fairly recently and also deeper more mature water which has recently been upwardly displaced. By turbulent, lateral mixing some of this deeper water of the Irminger current should become incorporated in the outwardly flowing cold, heavy bottom current. On a smaller scale similar events should have occurred over the Iceland-Faeroe Rise (Cooper, 1955).

The dominant characters of the waters which result are their high density, low temperature and low content of silicate.

After passage through the Denmark Strait the temperature of the Norwegian Sea water, though remaining relatively low, increased by more than

1° C. This has come about by admixture with other deep waters. Some of this is Iceland-Faeroe water which has navigated the eastern slope of the Reykjanes Ridge and turned into the western basin in 50–52° N. latitude. This is comparatively young, though itself subjected to mixing with older water during its journey. However, much of the admixing water will already have circumnavigated the deep North-western and North-eastern Atlantic Basins in an anticlockwise sense at least once. All of these contribute to the water which comes to underlie the enormous homogeneous mass of water around Southern Greenland already discussed. It should have acquired an apparent carbon-14 age likely to be measured in centuries; this although its dominant component had been calved only a few years earlier in the Denmark Strait or Iceland-Faeroe Channel.

On this view there is constant and considerable recycling of water around the deep basins of the North Atlantic. On each cycle the waters are rejuvenated by a proportion of relatively young Norwegian Sea water but the process is never complete. This eddying or recycling involves water which is getting ever older. The currents which may be measured either directly or by dynamical calculations are the sum of movements of water which has newly sunk and of water which is being recycled. Moreover, all three dimensions of space have to be considered.

In the course of this circulation of the deep North Atlantic, deep water from south of the Equator will become incorporated. In its turn the South Atlantic deep water will have acquired parcels of water from Antarctic, Pacific and Indian Oceans. The simplest interpretation of the world distribution of nutrients suggests that these other oceans have a much more lethargic deep circulation than the Atlantic, and that their carbon-14 age will prove to be much higher.

Analyses of carbon-14, therefore, present the oceanographer with a very powerful weapon for attacking this complex problem.

THE PUBLISHED DATA FOR $^{14}CO_2$ IN THE NORTH ATLANTIC

Let us now examine the data (Table I) published by Kulp (1953*a*, *b*). That the four surface waters are recent is to be expected. The first three are far from regions of sinking. The fourth is about 300 miles south-east of Cape Farewell, Greenland. However, the nearby 'General Greene' station 1994 worked by Soule & Graves (1937) on 3 August 1935 at 54° 47′ N., 41° 52′ W. does not suggest that the region is one of sinking deeper than 1000 m at most. None, therefore, gives a clue as to what composes the North Atlantic deep water at its places of origin.

The deep sample from the Norwegian Sea is of much interest. Helland-Hansen & Nansen (1909) suspected that this deep water might be of great age, a view not supported by the high oxygen content. Kulp's result indicates that water at 3182 m north-east of the Faeroes cannot have an 'age' exceeding 500 + 200 (= 700) years. It may well be very much less. Necessarily there must be an escape of water with density exceeding σ_t 28·0.

The sample at 58° 19′ N., 32° 57′ W. on the western slopes of the Reykjanes Ridge at 1800 m depth was well placed. The water thereabouts is composite in origin. The 'dominant' water which gives the water its salinity inversion is young and has come from the Iceland–Faeroe Ridge contouring around the slopes of the Reykjanes Ridge. From the results of the research vessels *Dana*, *Atlantis* and *Meteor*, especially from oxygen determinations, it is possible to conclude that there is also a slow deep drift of old, oxygen-poor water from the South Atlantic along the western slopes of the Mid-Atlantic Ridge. This takes place mostly at a depth considerably greater than 1800 m. Nevertheless, in the western basin north of 52° N., upward displacement and homogenization should be a very vigorous process at all depths, accounting for the large apparent age found for the 1800 m sample.

Water from this position continues to move northerly and then westerly until it is forced by the configuration of the sea bed to move south-west over and alongside the cold heavy water sinking from the Denmark Strait. Lateral and vertical mixing between the two very different parallel water masses must then occur. One result should be a rapid 'maturing' of the heavy water from the Denmark Strait on its way to form North Atlantic Deep Water.

The sample or samples from the southern tip of the Rockall Bank are not well placed. The water thereabouts has a highly characteristic temperature-oxygen relationship which suggests either a rapid increase in 'age' from a depth of about 2300 m towards the bottom or that the consumption of oxygen in the deep ocean is largely confined to the bottom and the water immediately above it. The organic carbon being oxidized by this process may have lain on the bottom for very many years. When transferred to the water by oxidation and upward mixing, it would be expected to impart a fictitiously high age to the water.

These several arguments suggest that whilst we are building up our knowledge of its distribution it may be wiser to report $^{14}CO_2$ in concentration units (per unit weight of carbon dioxide or per unit volume of water) which make no tacit assumptions about history.

Again, the significance of a determination of carbon-14 on a sample of sea water cannot be assessed unless the content of carbon-12 dioxide and everything else that has been discovered about the water is published. Date, depth, records of current, temperature, salinity, oxygen, nutrient salts and pH need to be reported or, alternatively, a reference to where these have been published and discussed should be given. No sound conclusions on age of water masses may yet be drawn.

Development

There is a strong case for directing effort to atmosphere and ocean in places and on occasions where conditions are likely to be extreme. Knowledge of extreme conditions should help evaluation of cases which are more average. Unfortunately, many of the places where extreme conditions may be expected are difficult of access.

The Atmosphere

Apart from places where coal and oil are burnt in large quantities, the highest concentrations of $^{12}CO_2$ are likely to occur over tropical areas of strong upwelling during quiet weather. Upwelling in the Pacific is likely to bring up more $^{12}CO_2$ than upwelling in the Atlantic. The upwelling waters off the desert coast of Peru are likely to produce the highest natural concentration of $^{12}CO_2$ in the air above to be found in the world. However, there is no reason to believe that $^{14}CO_2$ should there be exceptionally high. The $^{14}CO_2/^{12}CO_2$ ratio of air over upwelling water might be best determined during moderate south-easterly winds at a place on the coast of Peru in about 9° S. lat. The fetch of the air over upwelled water would then be several hundred miles.

Vegetation will assimilate all isotopes of carbon. The partition ratios for the several isotopes will be slightly different from the concentration ratios but within the limits of error of our present study, these differences may be ignored.

Areas of strong coastal upwelling are usually bordered by desert continental land. Even so the ratio of ^{14}C to ^{12}C in such vegetation as exists at places like Walvis Bay and the Peruvian guano zone is worthy of examination. To obtain samples it would be worth while to grow plants in pots. The ratio may be markedly lower than average. Moreover, in such places a strong seasonal variation in the isotopic ratio of the carbon assimilated may exist (cf. Currie, 1953), low at seasons of strong upwelling, average when upwelling is in abeyance.

To contrast with such samples, others for analyses of $^{12}CO_2$ and $^{14}CO_2$ are needed from (*a*) the bitterly cold air which stagnates in winter over Siberia at places like Verkhoyansk, Oymyakon or Yakutsk. (Here is an area likely to be particularly favourable for transfer of $^{14}CO_2$ from stratosphere to troposphere), (*b*) air which has sojourned for some time over Antarctica or over the Arctic sea ice, (*c*) air from these sources and from temperate latitudes which has blown for hundreds of miles over areas of oceanic sinking.

The Ocean

The following are needed:

Samples of water from outstanding regions of upwelling such as the Peruvian coast, and from the thick fast currents which set away from these coasts under the influence of the trade winds.

Samples from outstanding areas of sinking, such as the Norwegian Sea south of Jan Mayen, the oceanic area around Southern Greenland and the areas of formation of Antarctic bottom water.

Samples from precisely defined places where key processes in the circulation of the oceans are suspected of occurring. The Denmark Strait may be one such place and key positions are: (*a*) North of the Sill where the current at 200–500 m is flowing towards the south-west (say 68° 30′ N., 24° 40′ W.). (*b*) South-west of the sill crossing the meridian 27° 45′ W. between 66° 10′ and 66° 35′ N. latitude where on occasion the bottom current is believed to run fast on the continental terrace and parallel to the coast for the next 70 miles (130 km). In some years this position is overlain by ice. A more accessible position some miles to the south-east is unlikely to sample the water in question. Since the outflow is likely to be intermittent, it would be essential to establish that the desired water was being sampled. The season from March to June would be most appropriate. (*c*) At 66° 10′ N., 30° 30′ W. in what appears to be a submarine canyon extending south-east from the fjord Kangerdlugssuak.

Living organisms

The ratio of $Ca^{14}CO_3$ to $Ca^{12}CO_3$ in shells and of $^{14}C/^{12}C$ in shore plants and animals from the special areas listed above should be of interest. There should be a considerable difference between shells and organisms from, say, Spitzbergen or Jan Mayen on the one hand, and Peru or South-west Africa on the other.

SUMMARY

The carbon-14 method for determining the age of deep oceanic water gives ages much higher than are suggested by physical and chemical oceanographic observations. The discrepancy arises from tacit assumptions as to the age and nature of water masses and from peculiarities of the cycles of $^{12}CO_2$ and $^{14}CO_2$ in nature. These are:

(1) When surface water is under or over saturated to the same extent with oxygen or $^{12}CO_2$ equilibrium between atmosphere and ocean is much more rapidly restored with oxygen. An approach towards equilibrium which oxygen may achieve in 1 day requires 10–17 years for carbon-12 dioxide. Carbon-14 dioxide should behave similarly to carbon-12 dioxide.

(2) When polar water is transported via the deep ocean to upwell in the tropics, heating causes the partial pressure of $^{12}CO_2$ to increase about three times. Upwelling water must, for physico-chemical reasons, be supersaturated with $^{12}CO_2$ with respect to the atmosphere. A compensating concentration gradient for $^{12}CO_2$ must therefore build up in the atmosphere between tropics and poles. The corresponding gradient for $^{14}CO_2$ should be different.

(3) Due to the vast masses of water in areas of oceanic sinking which have to be equilibrated for $^{14}CO_2$ with the atmosphere and the complexity of the processes, it is unlikely that a water mass which an oceanographer would consider newly born, will sink with a zero age on the present carbon-14 scale. The content of $^{14}CO_2$ in sinking waters needs to be determined empirically and used as the starting point of a carbon-14 time scale.

(4) In the deep North Atlantic basin there is much recycling of ever-ageing water. This is rejuvenated by descent of Norwegian Sea water from the sills of the Denmark Strait and the Iceland-Faeroe Rise.

The significance of existing measurements of carbon-14 on deep oceanic waters is discussed. No sound conclusions on age of water masses may yet be drawn from them. It may be wiser, for the present, to report $^{14}CO_2$ in concentration units which make no tacit assumptions about history. On any sample, submitted for carbon-14 analysis, all the standard oceanographic measurements need to be reported.

Attention is directed to places where the concentration of $^{12}CO_2$ and $^{14}CO_2$ in atmosphere and ocean are likely to be extreme. Determinations in such places should hasten the assessment of determinations where conditions are more average.

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THE GROWTH RATE OF *CHTHAMALUS STELLATUS* (POLI)

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(Text-figs. 1 and 2)

Detailed data have recently been given on the growth rate, under varying conditions, of the common barnacles, *Balanus balanoides* (L.), *B. crenatus* Brug. and *B. balanus* (L.) da Costa (Barnes & Powell, 1953; Barnes, 1955; Barnes & Barnes, 1954). This work has now been extended to *Chthamalus stellatus* (Poli). Some observations have been made on populations growing in their natural habitat, but most relate to animals growing under conditions of continuous immersion such as are obtained by exposure from a raft.

The Material and Methods

Pieces of rock on which adult barnacles were growing or on which young spat had recently settled were detached from the shore and fastened to panels that could be mounted on frames for raft exposure. With few exceptions (see later, p. 357) all the material was taken from within the well-marked *Chthamalus* zone, which at Millport forms a narrow band lying above *Balanus balanoides*. On each piece about ten individuals were selected for measurement and their unrestricted growth ensured by the removal of the remainder, together with any other sedentary forms. At about monthly intervals the pieces of rock were brought into the laboratory and, in accordance with previous practice, the length of the base of each individual barnacle along its rostro-carinal axis measured. For the smaller individuals a binocular microscope with scaled ocular was used; the larger animals were measured with a vernier measuring microscope. At each inspection the stones were thoroughly cleaned to remove newly settled animals and, as far as possible, all algal growth. The observations have extended over a period of 2 years; during this time several sets of exposures have been made, of which seven are shown in Fig. 1. It should be pointed out that the irregular outline of *Chthamalus*, and the fact that the measurements have been made on individuals growing on rough natural sandstone, has led to rather less accurate results than with the species previously studied; the latter were usually grown from spat settled directly on glass or plastic panels.

For every set, the mean length of the selected individuals was calculated at each inspection; these values, together with the mean specific growth rates (the increase in length per unit length per day) are shown in Figs. 1 and 2.

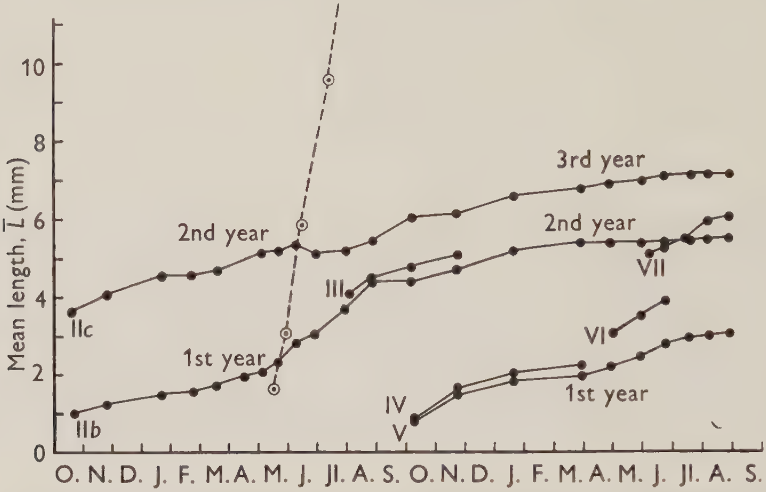

Fig. 1. The growth of *Chthamalus stellatus* over several seasons under conditions of total immersion. The mean lengths of each set at each date of inspection are shown. The growth of *Balanus balanoides* is shown for comparison.

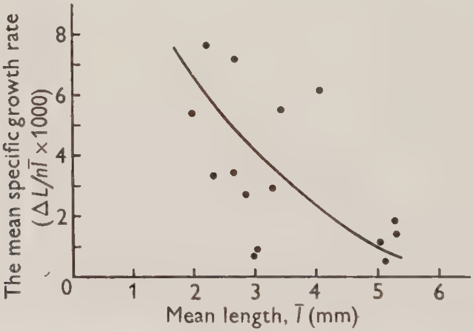

Fig. 2. The mean specific growth rate × 1000 of *Chthamalus stellatus* under conditions of total immersion for growth during the summer months, up to 6 mm length.

THE RESULTS

At Millport *Chthamalus stellatus* produces a number of broods during the summer months, but only that of the late summer and early autumn leads to a successful spat-fall. As is evident from the figures some growth takes place, under raft conditions, during the autumn and early winter following settlement, but becomes virtually negligible in the late winter months. After

7 months' growth, spat settled in September has reached a mean size of some 2·0 mm by the following April. During the late spring and summer of the first year, growth is renewed and at a greater rate, to fall off again in the autumn months. Twelve months from settlement a size of 3–4·5 mm has been reached. In the second year growth is slower, but the same pattern is repeated, although the contrast between summer and early winter growth is less striking. The same pattern is evident in all the series, but the uniformity from one series to another is less than with some other species. A length of 6–7 mm is reached by the end of the second year. The experiments were not continued beyond 2 years, but it would seem that those individuals, of maximum size some 10–15 mm, found on the shore, are at least 4 years old.

There is some evidence that for any size-group there is an enhanced growth rate directly after the change to conditions of total immersion; the initial parts of the growth curves are somewhat steeper than the central parts when animals of the same size and when growth at the same part of the year is considered. (Compare, for example, the appropriate parts of Series II*b* and VII, II*b* and II*c*, Fig. 1.) Further evidence that a sudden change to conditions of continuous immersion leads to an enhanced growth rate was obtained by following the growth, when transferred to raft conditions, of individuals which had been maintained at known levels on the shore for many months. These experiments were made possible through the kindness of Mr J. Connell, who allowed some of his experimental blocks to be used.

Table I. Comparison of the Growth of *Chthamalus stellatus* under Complete and Continuous Immersion after Transfer from Different Habitats

	Mean length in mm on		Increase in mean length in 2 months (mm)
Conditions prior to total immersion	20. vi.	26. viii	
High-tide level	3·1	4·9	1·8
Mid-tide level	3·6	4·0	0·4
Between mid- and low-tide level	2·3	2·9	0·6
Continuous immersion from settlement	2·8	3·0	0·2
Series VII, high-tide level	5·2	6·0	0·8
Series II *b*, continuous immersion from settlement	5·4	5·5	0·1

It is evident from Table I that when animals are transferred from the upper part of the shore to conditions of constant immersion, the initial growth rate is greater than that of similar individuals—of the same size and at the same time of the season—which have been completely immersed since settlement. Further, this enhancement of the initial growth rate is most marked with animals taken from the upper levels of the shore. This has not been demonstrated for *Balanus balanoides*; there is no evidence from the results so far available that it occurs in the sublittoral *B. balanus* on transference from the natural habitat to raft conditions (Barnes & Barnes, 1954). The capacity to react rapidly to immersion is clearly of importance to an intertidal animal since it will ensure maximal feeding when it is covered by tide. However, continued immersion, at least with *Chthamalus stellatus*, results in the establishment of a steady normal growth rate and, presumably, metabolic activity. This would appear to be another example of acclimatization (see Bullock, 1955, for a recent discussion), a return being made, under the new conditions of the environment, to the general metabolic level of the animals under 'equivalent' intertidal conditions.

The mean specific growth rate (Fig. 2) during the growing season decreases with increasing size. Due no doubt in part to the difficulties of making accurate measurements (see p. 355) the spread of the results is greater than was found for other species; there is, however, some evidence (Hatton, 1938) that growth in *Chthamalus* does vary considerably from year to year. The rate of change of growth rate with length is greater than for *Balanus balanoides* when both sets of measurements are expressed in terms of maximum size attained during the first growing season.

COMPARISON WITH OTHER RESULTS

The literature contains very few references to the growth rates of *Chthamalus stellatus*. Hatton (1938), working in the St Malo area, found that growth in an exposed situation was most rapid at the lower tidal levels during the first 9 months after settlement; at mid-tide level a length of 4·1 mm, and at a position slightly above high-water neaps 2·6 mm was attained in the same period. There was considerable variation from year to year; for example, only 2·7 and 2·0 mm were reached at these same levels in another year. During the second season the mid-tide population grew very little, but that at the higher level increased in size from 2·6 mm to between 4 and 5 mm. He could detect no significant seasonal difference in growth rates, but this may have been a consequence of the methods he used for these very slow-growing barnacles. (The growth of individual barnacles was not followed and he sometimes obtained a decrease in mean size in his samples.) In less exposed localities Hatton found that the more rapid growth at his lower levels was continued into the second season. Moore & Kitching (1939) have suggested that there was some unfavourable influence at work at the lower levels examined by Hatton which became progressively more effective with increasing age of the barnacles. These workers found that at Plymouth growth over a period of 4 years was always greater at the lower levels, even though the two levels examined were only about 1 m apart. (These authors give sizes in terms of (length + width)/2; for the purposes of comparison this will give somewhat smaller values than length alone.) At their upper level (+1·36 m) the mean size at 12 months from settlement was 2·0 mm, and after 24 months 3·7 mm; at their lowest level (+0·41 m) the sizes reached, during the same period, were 3·9 and 5·0 mm. Similar growth rates have been obtained for shore populations at Millport. The growth rates under conditions of continuous submersion are clearly very similar to those encountered on the shore. It would seem, therefore, that the harmful effect of continuous immersion postulated by a number of workers to explain the restriction of *Chthamalus stellatus* in some places to the highest levels of the intertidal zone is not warranted: it is not the necessity of alternate wetting and drying which ever limits *Chthamalus* to the upper levels of the shore in any region.

Discussion

It is evident from these results that *Chthamalus stellatus*, kept under the stated conditions on a raft, grows at a much slower rate than *Balanus balanoides* under the same conditions (Fig. 1); the latter species virtually reaches its maximum size in the first season's growth, i.e. some 6 months after settlement. Equally striking is the small effect that continuous immersion has on *Chthamalus* in comparison with its growth rate on the shore. At similar sizes the growth rate under the experimental conditions is greater than on the shore either at high or low levels, but in contrast with *Balanus balanoides* the difference between the growth rates is much less striking. It has been shown that, in the latter species, whilst increased opportunity for feeding contributes to enhanced growth rates under raft conditions, the fact that the experimental panels are kept free from algal and other growths is of paramount importance (Barnes & Powell, 1953; Barnes, 1955). The smaller effect in *Chthamalus* may be due in part to the fact that the comparison of growth rates has been made with individuals which in their natural habitat are subject to a much smaller depressing effect of algae. However, it is well known that *Chthamalus* is found most abundantly in wave-swept positions; the advantages to be gained in feeding opportunity and freedom from algal growth under the experimental conditions may, therefore, be offset in this species by the lack of violent water movement under conditions of complete submergence.

C. stellatus and *Balanus balanoides* are two competing barnacles in the intertidal region; the former is a tropical-Lusitanian form and the latter Boreo-Arctic in its distribution and both are at the limit of their distribution in the British Isles. In general, towards the north, where conditions are more favourable for the breeding of *Balanus balanoides* when the two species co-exist, *Chthamalus stellatus* as a major community is restricted to a level above that of the former species. Towards the south, as conditions become more favourable for the breeding of *Chthamalus*, it is found lower down the shore, extending into the *Balanus* zone, which it may ultimately replace. Southward & Crisp (1954) have stressed that since both species are at the limits of their distribution in Britain, they are, therefore, very sensitive to changes in the environment. This is reflected in the comparatively rapid changes which take place in the total and relative numbers of the two species at different levels of the shore. Changes favourable to *Chthamalus* result not only in an increase in its numbers at the upper levels but also in its spread down the shore. Many factors play a part in any competition between these two species, but ultimately many of these become effective through the difference in growth rate in the struggle for intertidal space. *Balanus balanoides* spat, settled on experimental rocks on which *Chthamalus* was growing and exposed on the raft, rapidly overgrew the latter; a 6-months-old *Chthamalus* settlement (2 mm long) was obliterated in a *few weeks* by a moderate spat-fall of *Balanus*, and full-grown *Chthamalus* (9–15 mm) were completely overgrown in 2 months. Where conditions are favourable to the breeding of *Balanus*, i.e. towards the north and where there is, therefore, a good spat-fall each spring, *Chthamalus* settled in the previous autumn has little chance of survival on the lower parts of the shore (although occasional individuals may be found). The growth rate of any which do survive is perhaps even more affected by algal cover than is that of *Balanus balanoides*. *Chthamalus* becomes, therefore, as a major community, restricted to the upper level of the shore, usually above that of *Balanus*, where it survives presumably by its greater capacity to withstand desiccation. As one proceeds southwards conditions become more marginal for the breeding of *B. balanoides* and more favourable for *Chthamalus*; the annual spat-fall of the former becomes progressively reduced and the latter can maintain itself even in the lower parts of the shore. Any change in the environment, from whatever cause, which affects the breeding, annual spat-fall and survival after settlement of the two species will be reflected in this competition for space which in turn is intimately related to the growth rates.

SUMMARY

1. Data are presented on the growth rate of *Chthamalus stellatus*; growth is slow in the first few months after settlement, virtually ceases in the winter and is renewed during the following spring.

2. Directly after transferring from the shore to raft conditions of constant submersion there is an acceleration, at all sizes, of the growth rate. However, acclimatization with a return to the slower rate soon takes place.

3. Growth rate under raft conditions is not greatly different from that on the shore. This is in marked contrast to *Balanus balanoides* and is ascribed in part to the relative lack of smothering algae in the upper parts of the shore so that there is less contrast in the conditions after transfer.

4. The effect of growth rate on competition between *Chthamalus stellatus* and *Balanus balanoides* for space in the intertidal zone is stressed.

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ON THE SCYPHOMEDUSAE *NAUSITHOË ATLANTICA* BROCH AND *NAUSITHOË GLOBIFERA* BROCH

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(Plate I and Text-figs. 1–6)

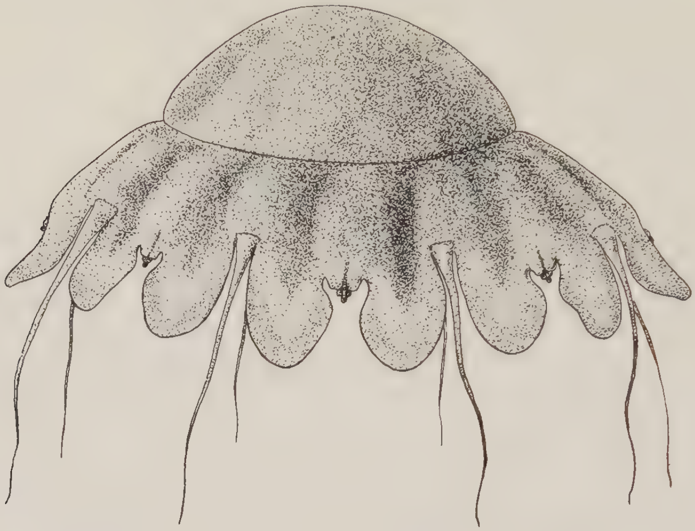
Broch (1913) described two new species of the coronate scyphomedusa *Nausithoë* from the North Atlantic, *N. atlantica* and *N. globifera*. Bigelow (1928, p. 498) was inclined to think that these two species might finally prove to represent extreme variants of the one species *N. rubra* Vanhöffen, though a much larger series of specimens in a better state of preservation would be needed to give a definite decision. Kramp (1947, p. 46) considered that *N. globifera* at any rate was a good species.

I have recently seen four specimens, two of which agreed with Broch's description of *N. atlantica* and two with that of *N. globifera*. Examination of these medusae leaves no doubt about the correctness of Broch's identification. These specimens were collected in a 2 m stramin ring trawl by R.V. *Sarsia*, the two *N. atlantica* (35 and 25 mm in diameter) at 47° 12′ N., 7° 40′ W. on 21 July 1955 with 450 fathoms of wire out, and the two *N. globifera* (22 and 11 mm in diameter) at 46° 49′ N., 5° 44′ W. on 6 September 1955 with 500 fathoms of wire out (vertical haul).

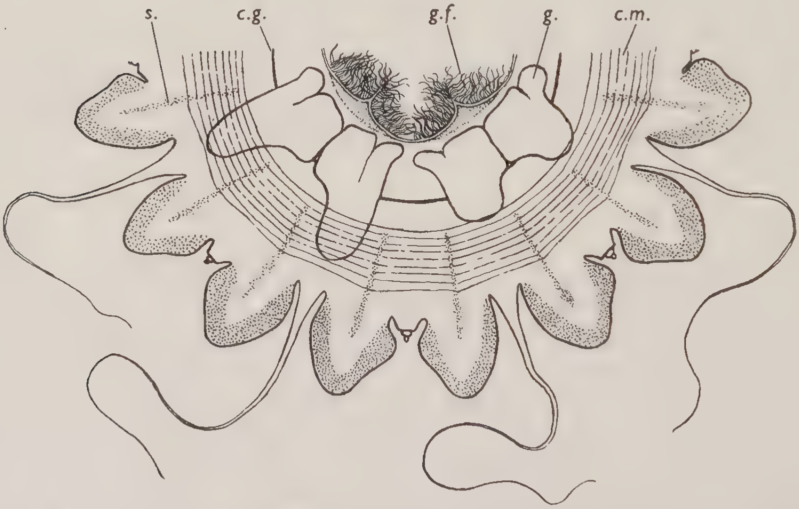
The two species were at once distinguishable both by their shape and coloration, and a closer examination showed other differences.

Nausithoë atlantica

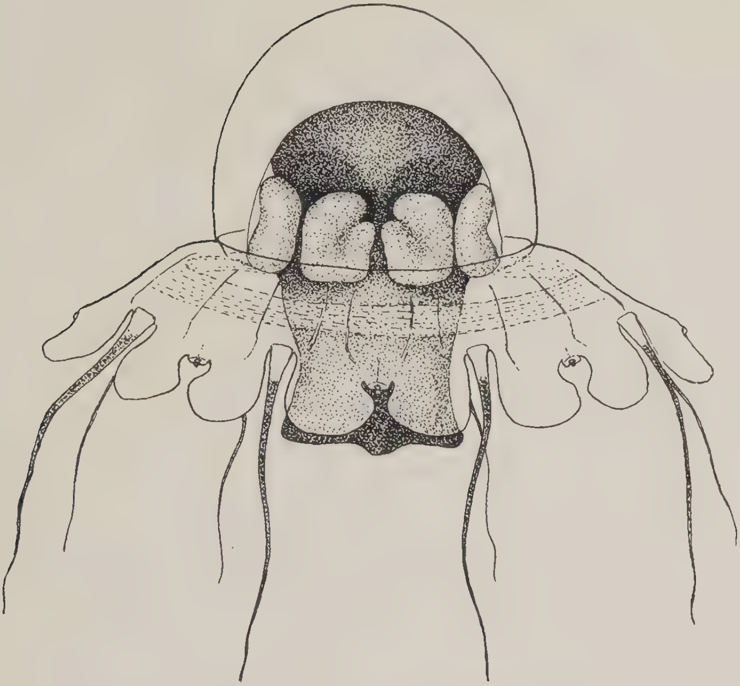
The largest specimen of *N. atlantica* recorded by Broch was 28 mm in diameter; my largest specimen is about 35 mm in diameter, the diameter at the coronal groove being 19 mm (Text-fig. 1; Pl. I). In this specimen three of the gonads are the same shape as drawn by Broch (1913, pl. I, fig. 2), but five of them have reached a further degree of development not yet recorded. The central part of each gonad is extended towards the umbrella margin so as to be between the septa and ending only a short distance from the base of the marginal tentacle (Text-fig. 2). The only other respect in which my specimens differ from Broch's description is that the pedalia are all about the same length. Broch stated (and his pl. I, fig. 1, shows) that the rhopalar pedalia were shorter than the tentacular pedalia. In my specimens all pedalia are approximately equal in length; if anything the rhopalar pedalia are slightly the longer, and this is rather to be expected because the distance between the coronal groove and the rhopalium is longer than that between the coronal groove and the base of the marginal tentacle.



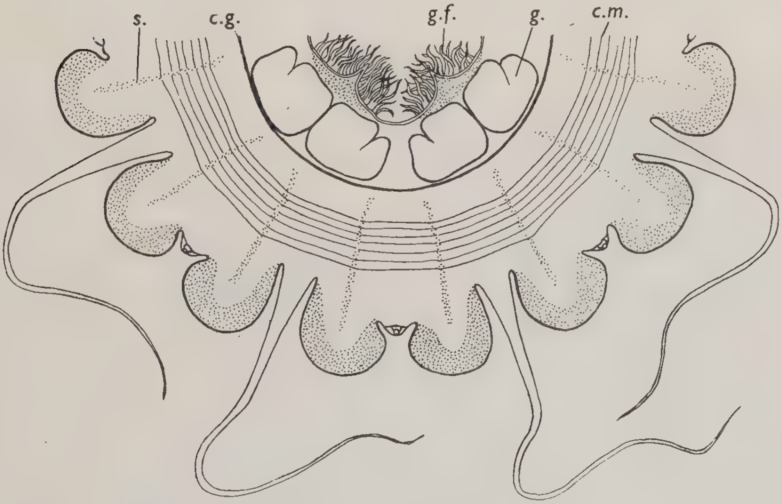
Text-fig. 1. *N. atlantica.* Adult medusa, 35 mm in diameter.



Text-fig. 2. *N. atlantica.* Semi-diagrammatic view of subumbrella with buccal walls of stomach cut away. The stippled areas indicate regions of fusion of upper and lower endoderm surfaces. *c.g.*, coronal groove; *c.m.*, coronal muscle; *g.*, gonad; *g.f.*, gastric filaments; *s.*, septum.



Text-fig. 3. *N. globifera.* Adult medusa, 22 mm in diameter.



Text-fig. 4. *N. globifera.* Semi-diagrammatic view of subumbrella with buccal walls of stomach cut away. Details as in Text-fig. 2.

In my specimens the exumbrella surface is considerably pitted; these pits appear to be due to the pressure of radiolaria into the jelly, presumably during the process of collecting. I have not shown them in Text-fig. 1, but they can be seen in Pl. I. The marginal lappets also were mostly frayed, though there was sufficient intact margin to supply evidence of their true outlines which I have shown in Text-fig. 1. There are twenty or more gastric filaments on each phacellus, making over 160 in all, and each filament arises singly.

Sections of the gonad show that the mature ova are each completely enveloped by a layer of densely packed oval bodies lying immediately beneath the egg membrane. These bodies, the largest of which are about 12–14 μ in length, appear first in the smallest ova as minute granules. As the ova increase in size so the granules grow and move towards the periphery. The bodies have a central core, but no other structure is visible.

The colour of the whole medusa, when freshly preserved in formalin, is chocolate red identical with that of *Paraphyllina ransoni* (Russell, 1956). As in that species, sections show that the coloration is limited to the mesogloea and forms a dark membranous layer immediately beneath the endoderm. The brownish yellow colour also occurs faintly throughout the whole mesogloea so that the umbrella jelly has not the striking transparency of that of *Paraphyllina ransoni*. As in the latter species the stomach walls are especially densely coloured, both under the endoderm and the ectoderm, and the mesogloea of the marginal lappets is coloured throughout. The whole rhopalium is coloured, and the underside of the hood is especially dark (Text-fig. 5). After storage in formalin and sea water for several months the whole medusa becomes much more transparent and dark amber in colour.

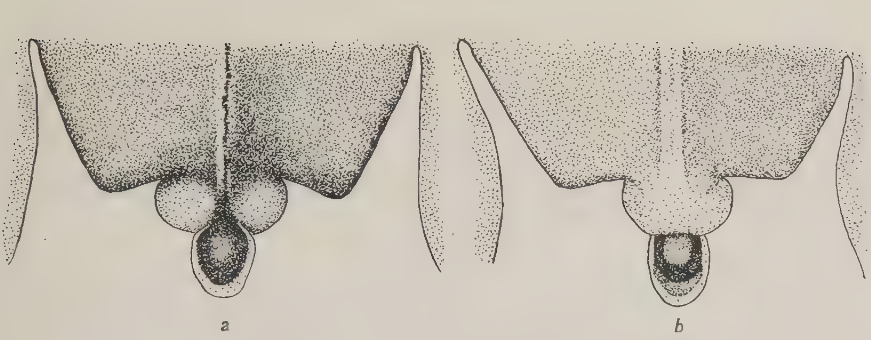
Nausithoë globifera

The largest specimen of *N. globifera* recorded by Broch was 17 mm in diameter; my largest specimen, which was perfectly preserved, is 22 mm in diameter, the diameter at the coronal groove being 10 mm (Text-fig. 3 and Pl. I).

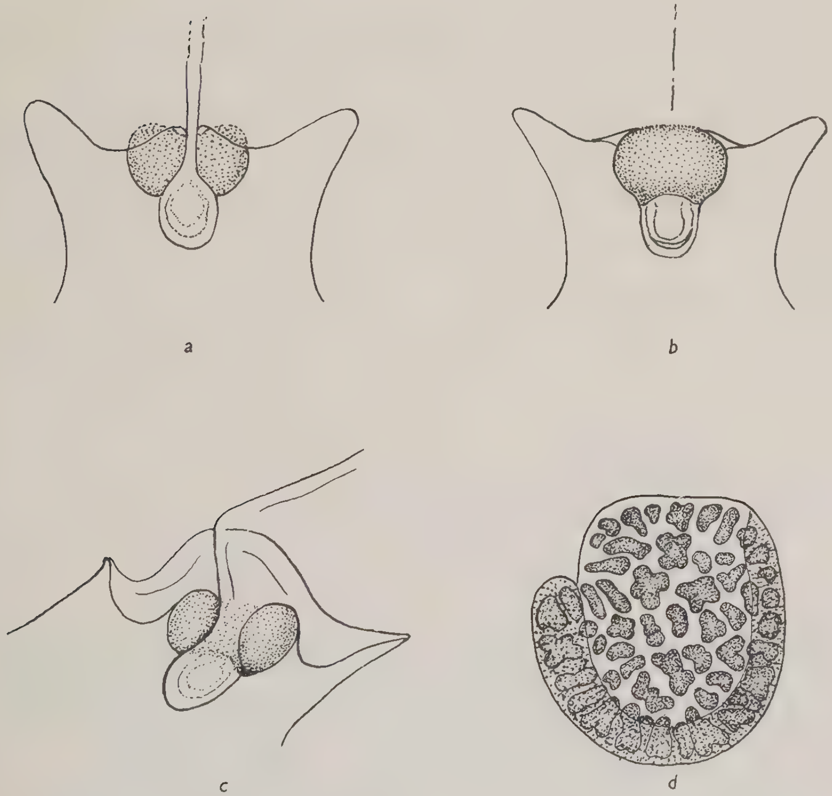
My specimens agree in every respect with Broch's description.

The gonads in the smaller specimen, a male, are shield-shaped, and their interradial edges are folded inwards towards the subumbrella. This fold continues round the lower margin of the gonad to within about one-third of the upper end of the perradial side. At the latter point the fold ends and joins the main body of the gonad at its inner edge (Text-fig. 6*d*). In the larger specimen, in which the gonads are further developed, this sudden reduction in the width of the upper part of the gonad makes it look as though there is a protruding thumb when viewed from the exumbrellar side (Text-fig. 3).

The gonads in the smaller specimen were white except for fine flecks of pigment, and they showed in striking contrast against the dark colour of the



Text-fig. 5. *N. atlantica.* *a*, *b*, exumbrellar and subumbrellar views respectively of rhopalium.



Text-fig. 6. *N. globifera.* *a*–*c*, exumbrellar, subumbrellar, and lateral oblique views respectively of rhopalium; *d*, male gonad of specimen, 11 mm in diameter seen from subumbrellar side.

stomach. In the larger specimen the gonads were coloured reddish brown, but they were still very obvious against the dark stomach wall.

The gastric filaments, of which there are about ten in each phacellus, making eighty or more in all, are mostly simple, but on any one phacellus there may be two or three roots which each give rise to two to four filaments.

The rhopalia are bent at right angles so that they are hidden from sight when viewed from above but can be seen in a lateral view.

The whole medusa is perfectly transparent except for the stomach, the marginal tentacles, and part of each rhopalium. The colour of the stomach was deep purple-red when freshly preserved in formalin and sea water, being darkest over the summit. The colour of the marginal tentacles was a more orange red; a short portion at the base of each tentacle was uncoloured. Only the ventral bulb of the rhopalium was coloured and this only faintly (Text-fig. 6).

Sections show that the coloration is limited to a membranous layer lying in the mesogloea immediately beneath the endoderm of the stomach. In the buccal walls of the stomach the whole mesogloea is coloured. There are in addition pigment granules present in the endoderm cells lining the whole stomach, and in the walls of the gonads. The mesogloea of the marginal tentacles and gastric cirri is also coloured.

The essential differences between the two species may be summarized as follows:

	N. atlantica	*N. globifera*
Shape	Somewhat flattened	High domed
Colour	Whole medusa chocolate red; coloration in mesogloea only	Umbrella transparent; stomach deep purple red and marginal tentacles orange red; coloration in both mesogloea and endoderm cells
Gastric cirri	About 160 in number, generally single	About 80 in number, several compound
Gonads	Situated mainly in marginal disc region	Situated above coronal groove
Rhopalia	With large wedge-shaped flat basal cushion and exumbrella carina. Under side of hood and ventral bulb deeply coloured	With small high wedge-shaped basal cushion and exumbrella carina bent at right angles. Ventral bulb only faintly coloured

There is now sufficient evidence to prove that *N. globifera* Broch cannot be identified with *N. rubra* Vanhöffen. I think also that *N. atlantica* Broch must be regarded as a good species for which we now have an adequate description, and that it is not possible to decide whether it is the same as *N. rubra*.

My thanks are due to Captain C. A. Hoodless and the crew of R.V. *Sarsia* who collected the specimens; to Dr D. P. Wilson for the upper photographs in Pl. I; to Dr J. S. Alexandrowicz for the preparations and photographs in the lower half of Pl. I; and to Miss M. L. Weir for the preparation of sections.

SUMMARY

Specimens of the coronate scyphomedusae *Nausithoë atlantica* Broch and *N. globifera* Broch were caught in deep water off the mouth of the English Channel in July and September 1955 respectively.

These were well preserved and there is now no doubt that these are two distinct species.

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POSTCRIPT

Since the above account was written I have seen three more specimens of *N. atlantica* and seventeen of *N. globifera*. These were from samples collected in the 2 m. ring trawl, the details being as follows:

Date	Position	Diameter at coronal groove (mm.)
	N. atlantica	
20. vii. 55	47° 12′ N., 7° 40′ W.	Female, 9
7–8. ii. 56	46° 46′ N., 5° 47′ W.	Females, 12, 14
	N. globifera	
17. vi. 55	46° 49′ N., 5° 44′ W.	Female, 8
20–22. vi. 55	47° 12′ N., 7° 40′ W.	Females, 7·5, 8·5, 9, 10 Males, 8, 8, 9, 9·5, 10
7. ii. 56	46° 46′ N., 5° 47′ W.	Females, 6·5, 7, 7 Males, 6, 7·5, 10·5, 11

The only additional information obtained by examination of these specimens was:

N. atlantica: all the gonads may extend nearly to the bases of the marginal tentacles.

N. globifera: the thumb-like process is not so apparent in the female gonads as in the male gonads. Sections of the female gonads show bodies in the ova similar to those described above for *N. atlantica*.

In both species the mature eggs are white; and in both species the marginal tentacles are laterally compressed except close to the basal bulb, possibly an effect of preservation.

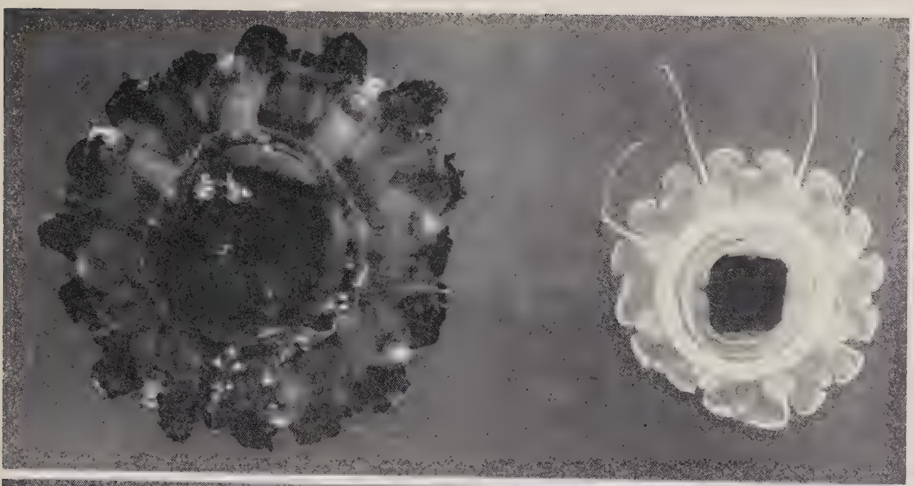
EXPLANATION OF PLATE I

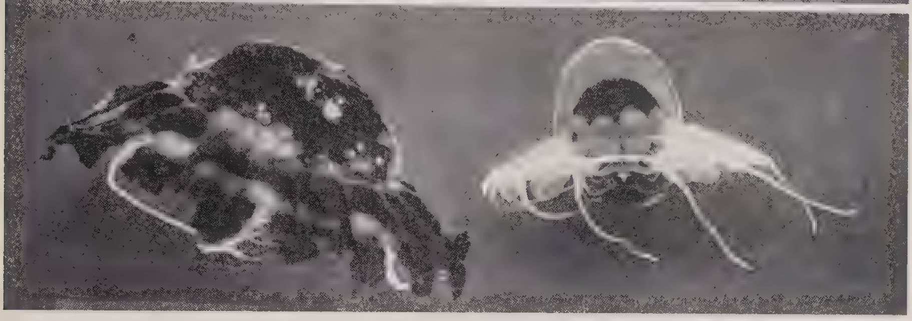
Left: *Nausithoë atlantica* Broch. Right: *N. globifera* Broch.

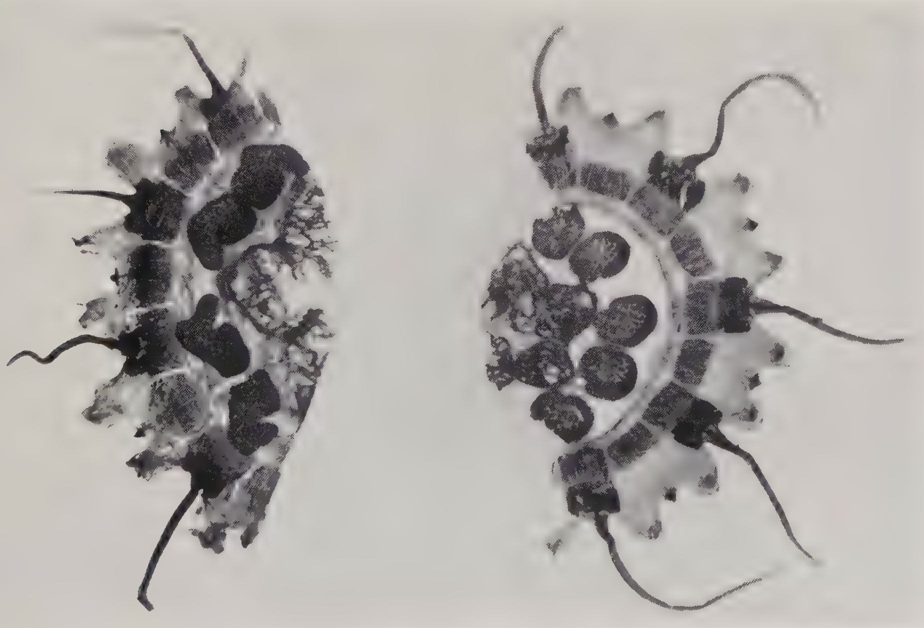
Above: exumbrellar and lateral views of freshly preserved specimens, × 1·7.

Below: sectors of umbrella bleached by Mayer's chlorine method, stained with borax carmine and mounted.

N.B. In the upper photographs of *N. atlantica* the gonads can be seen in places showing through the jelly. The white objects present on the dome of the umbrella are radiolarians which have become pressed into the jelly.







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ON A NEW MEDUSA, *AMPHINEMA KRAMPI* N.SP.

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(Text-figs. 1 and 2)

In a collection of plankton made by R.V. *Sarsia*, in a 2 m stramin ring trawl at 47° 12′ N., 7° 40′ W., on 21 July 1955 with 450 fathoms of wire out, there was a small anthomedusa which does not agree with any previously described species.

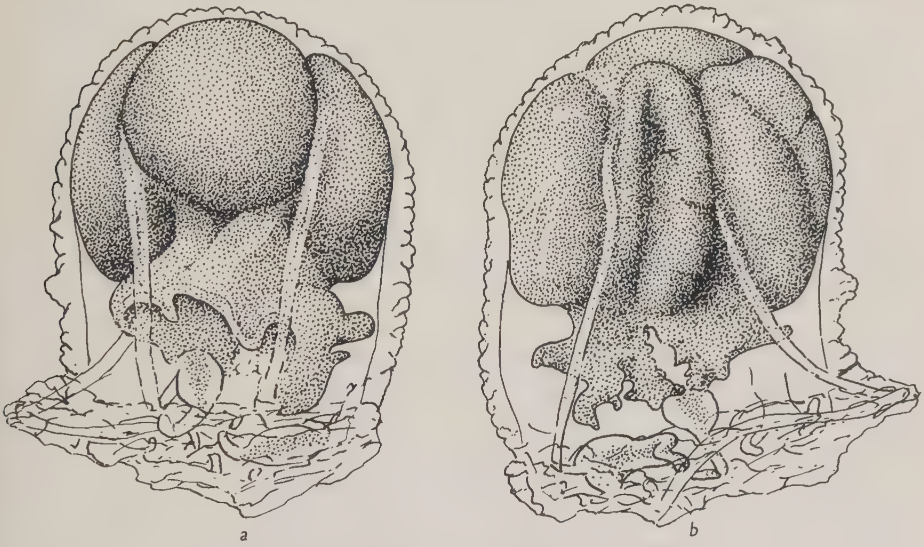

Fig. 1 *a*, *b*. *Amphinema krampi* n.sp.; the specimen has been drawn from both sides to show it as it actually looks in the preserved state.

The specimen is sufficiently well preserved to show its chief characters, and apparently belongs to the family Pandeidae. Its characters are such that it can be included in the genus *Amphinema*.

I have great pleasure in naming the medusa *Amphinema krampi* n.sp. in honour of P. L. Kramp, the world's leading authority on medusae.

Amphinema krampi n.sp.

The umbrella is bell shaped and higher than wide; the jelly is much wrinkled and contracted but would in nature have been fairly thick; there is no apical process. The stomach is cross-like in section and about two thirds the length of the subumbrellar cavity; it has no peduncle. The mouth is cruciform with four simple lips with a thickened margin and almost no crenulation. The four radial canals and ring canal are fairly broad; they have smooth margins; there are about twelve strands of cellular tissue running from each radial canal to the exumbrella surface, distributed along the length of each canal between the summit of the stomach and the level of the mouth lips. The gonads, which are male, form four simple cushions, one on each interradial surface of the stomach. There are two opposite perradial marginal tentacles with swollen elongated basal bulbs, and eight marginal tentaculae. There are no ocelli. The height of the umbrella is about 6 mm and it is about 4 mm wide. The colour of the stomach and gonads is rich reddish brown, and there is a core of brownish pigment in the ring canal extending into each of the two marginal tentacle basal bulbs; the marginal tentaculae are colourless.

Drawings of the specimen are given in Fig. 1 *a* and *b*. It can be seen that the umbrella jelly is shrunken and that the umbrella has been much contorted in the marginal region. Two of the gonads on one side have been squashed so that they are folded down their centres, and the mouth has been torn on that side.

Nevertheless it has been possible to reconstruct the medusa fairly accurately and a drawing of its probable appearance if it had been well preserved is given in Fig. 2.

As there is only one specimen I did not have sections made for microscopic examination. One or two details remain therefore undetermined.

The strands of cellular tissue running from the radial canals to the exumbrella surface are of special interest, and nothing like them has ever been recorded before. They appear as though they might be canals running from the radial canals to the exterior, but microscopic sections would be required to prove whether this were so.

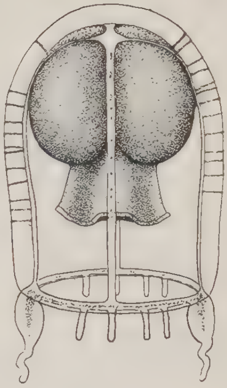

Fig. 2.

Amphinema krampi n.sp.: the medusa reconstructed as it would probably appear if well preserved.

The marginal tentacles appear to be typical of pandeid medusae and it is probable that they are hollow. The marginal tentaculae are uniformly covered with nematocysts. The distribution of the tentaculae around the margin of the umbrella is rather irregular. In one sector at any rate, one tentacula is perradial so that there are two in one interradius and one in the other. The distribution appears to be similar in the other sector, but as the radial canal is damaged on that side it is not possible to say for certain whether one tentacula is perradial. There is no indication that there are any missing tentaculae.

Owing to the dark coloration it was not possible to tell the sex except by breaking one of the gonads, when the specimen was found to be a male.

The following characters distinguish *Amphinema krampi* from other species in the genus: no apical process; cellular strands of tissue running from radial canals to exumbrella surface; four simple interradial gonads.

The specimen has been deposited in the British Museum (Natural History) and has been given the registered number B.M. 1956.1.10.1.

My thanks are due to Captain C. A. Hoodless and the crew of R.V. *Sarsia*, who made the collection in which the specimen was found.

SUMMARY

A single specimen of a new pandeid medusa was caught in deep water off the mouth of the English Channel in July 1955.

A description is given, and the species has been named *Amphinema krampi* n.sp.

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THE SIZE OF DIATOMS

III. THE CELL WIDTH OF *BIDDULPHIA SINENSIS* GREVILLE FROM THE SOUTHERN NORTH SEA

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(Text-figs. 1–5)

The present contribution deals with the measurements of fifty-two samples of *Biddulphia sinensis* collected by Hensen net hauls from the Ministry of Agriculture and Fisheries research ships *George Bligh* and *Onaway*, working in the southern North Sea between 1932 and 1938. With one exception in which there were only seventy available, the samples consisted of a hundred cells and the unit of measurement was that employed in Part I of this paper (Wimpenny, 1936). The dimension used was the greatest width (apical axis), and care was taken to see that the cells measured were lying flat, to give the true maximum of their elliptical cross-section. For convenience in tabulation and to avoid random fluctuations due to too small grouping of the data, the measurement units have been taken in pairs in the presentation of all the sea results, each arbitrary unit being equivalent to about 8 μ. The measurements from cultures, where the samples were sometimes fewer than a hundred, have been 4 μ units.

The positions of the stations, the mean widths, the population densities and the temperatures and salinities relating to the samples are given in Table I. The individual measurements from which this table has been compiled have been deposited at the Marine Laboratory, Plymouth, whence they may be obtained for consultation on request.

Observations made on cell width

A representative series of size-frequency samples covering the period 1932–38 has been plotted in Fig. 1. This may usefully be compared with Text-fig. 2 in Part II of this work (Wimpenny, 1946). It will be seen at once that the regularly recurring and persistent form of the size distribution shown by *Rhizosolenia styliformis* is absent in *Biddulphia sinensis*, where the width distribution in one year, or indeed on one cruise, may vary as much as it does throughout the whole period.

These big variations within the area of one cruise are, however, more explicable when related to the salinity of the surrounding water, and in Fig. 2 I have shown the surface salinities of two cruises in 1937 in relation to the

TABLE I. POSITION OF STATIONS AND OTHER DETAILS REFERRING TO THE DIAMETER MEASUREMENTS OF *BIDDULPHIA SINENSIS*

Date	Cruise	Station	Depth (fathoms)	Latitude N.	Longitude E.	t° C at 0 m	Salinity at 0 m	Mean diameter in arbitrary units	1000's per m³
26. x. 32	J	7	15	52° 55′	4° 00′	13·67	34·67	17·6	23·8
28. x. 32	J	17	15	53 45	6 07	12·57	33·40	16·7	30·3
7. x. 33	K	4	13	52 12	3 53	16·70	33·82	16·4	136·5
8. x. 33	K	8	17	53 06	3 02	16·70	34·66	17·4	351·7
8. x. 33	K	10	18	53 26	2 27	16·46	34·62	19·1	1417·2
19. x. 33	L	15	16	53 19	3 07	14·89	34·85	17·8	375·6
20. x. 33	L	22	17	53 45	2 35	14·32	34·73	17·9	6·1
28. viii. 34	J	35	18	52 03	3 15	18·12	34·60	20·1	0·6
28. viii. 34	J	36	28	52 03	2 11	17·03	35·07	14·5	1·8
27. ix. 34	L	24	27	52 00	2 11	16·80	35·09	14·4	470·4
27. ix. 34	L	26	16	51 48	3 15	17·21	34·81	16·4	146·2
28. ix. 34	L	29	14½	53 00	3 12	16·76	34·89	14·8	332·6
20. x. 34	N	2	30	52 20	2 28	15·07	35·10	15·6	300·9
22. x. 34	N	20	15	53 00	3 49	14·17	35·03	14·9	1284·4
1. xi. 34	O	10	24	54 27	4 40	12·74	34·82	16·7	787·5
3. x. 35	O	4	15	51 45	3 18	15·57	34·65	16·9	235·8
4. x. 35	O	11	22	53 54	3 03	14·35	34·62	14·8	1650·0
4. x. 35	O	12	19	53 36	2 21	13·98	34·60	15·6	783·9
25. x. 35	P	13	29	54 10	4 30	12·87	34·76	17·1	124·9
9. xi. 35	Q	24	25	53 44	4 09	11·73	34·83	16·3	428·6
24. xi. 35	R	6	24	54 54	5 17	10·30	34·81	18·9	231·8
27. ix. 36	N	19	17	53 30	2 57	16·12	34·11	20·6	146·2
27. ix. 36	N	20	14	53 30	3 53	16·47	34·40	19·1	166·1
29. ix. 36	N	29	7	51 31	1 24	15·84	34·50	18·1	870·0
29. ix. 36	N	30	14	51 29	0 52	15·39	33·56	18·2	490·5
6. x. 36	O	2	19	52 00	2 06	15·84	34·43	18·2	209·0
11. x. 36	O	20	22	53 17	2 25	14·31	34·28	19·1	2106·8
14. x. 37	M	24	32	54 07	2 25	14·37	34·85	15·4	27·5
15. x. 37	M	25	20	53 43	2 53	14·53	34·14	18·5	1234·1
15. x. 37	M	26	18	53 26	2 11	14·38	34·18	19·6	121·0
27. x. 37	N	4	10	52 42	4 23	13·11	32·03	20·8	138·9
27. x. 37	N	5	15	53 06	4 21	13·21	33.97	25·2	3·0
28. x. 37	N	12	13	53 35	2 14	13·26	34·84	15·9	16·0
28. x. 37	N	13	17	53 35	2 57	13·48	34·13	19·1	1890·0
14. xi. 37	O	8	16	53 32	3 42	11·35	34·14	20·0	165·0
15. xi. 37	O	12	10	53 32	2 20	11·47	34·52	20·9	339·0
15. xi. 37	O	16	17	53 57	2 23	11·77	34·83	16·9	197·5
15. xi. 37	O	17	24	53 58	3 06	12·02	34·65	19·1	384·8
15. xi. 37	O	18	19	53 58	3 47	11·77	34·43	20·1	481·9
16. xi. 37	O	19	21	53 58	4 20	11·69	34·00	19·6	20·6
16. xi. 37	O	21	22	54 23	3 32	11·29	34·93	15·4	547·3
16. xi. 37	O	23	17½	54 20	2 08	11·67	34·70	14·9	7·1
28. xi. 37	P	1	10½	53 32	2 20	9·60	34·48	19·6	384·0
28. xi. 37	P	2	17½	53 32	3 02	9·88	34·66	19·9	23·8
21. x. 38	P	5	13	52 48	4 01	14·39	34·63	25·5	339·8
21. x. 38	P	6	17	52 32	3 25	14·57	34·78	19·3	250·0
22. x. 38	P	7	18	53 14	3 35	14·19	34·88	15·3	28·6
23. x. 38	P	12	19	53 38	3 09	12·38	34·58	15·6	2·2
23. x. 38	P	13	35	54 00	2 34	13·19	34·66	17·1	81·8
11. xi. 38	Q	7	22	54 48	4 32	12·23	34·68	18·0	77·5
13. xi. 38	Q	19	25	57 12	7 38	11·50	—	16·7	420·5
14. xi. 38	Q	20	21	56 08	6 32	12·36	—	19·6	14·9

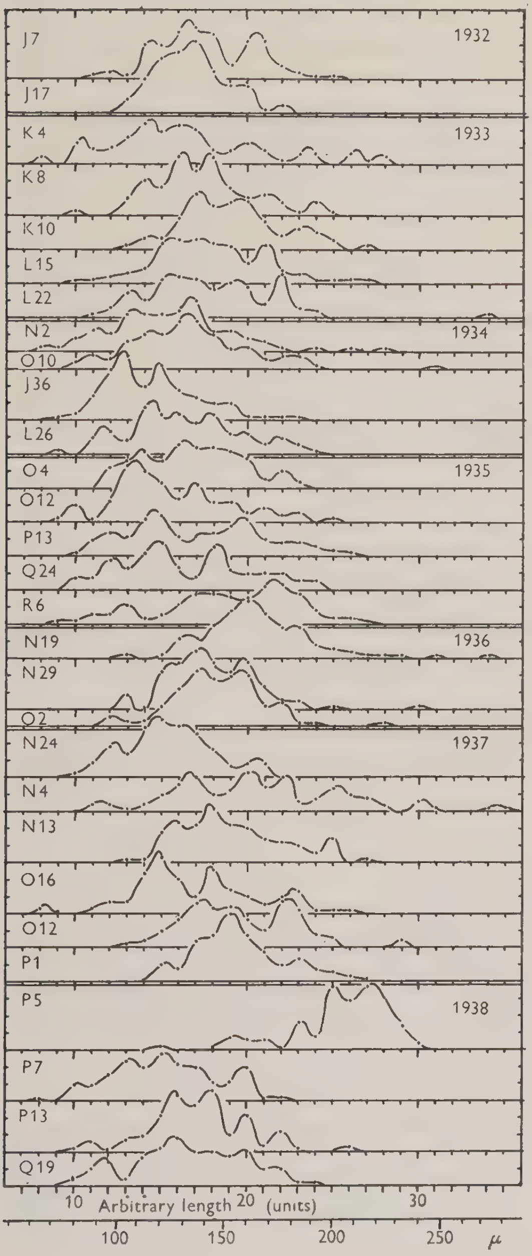
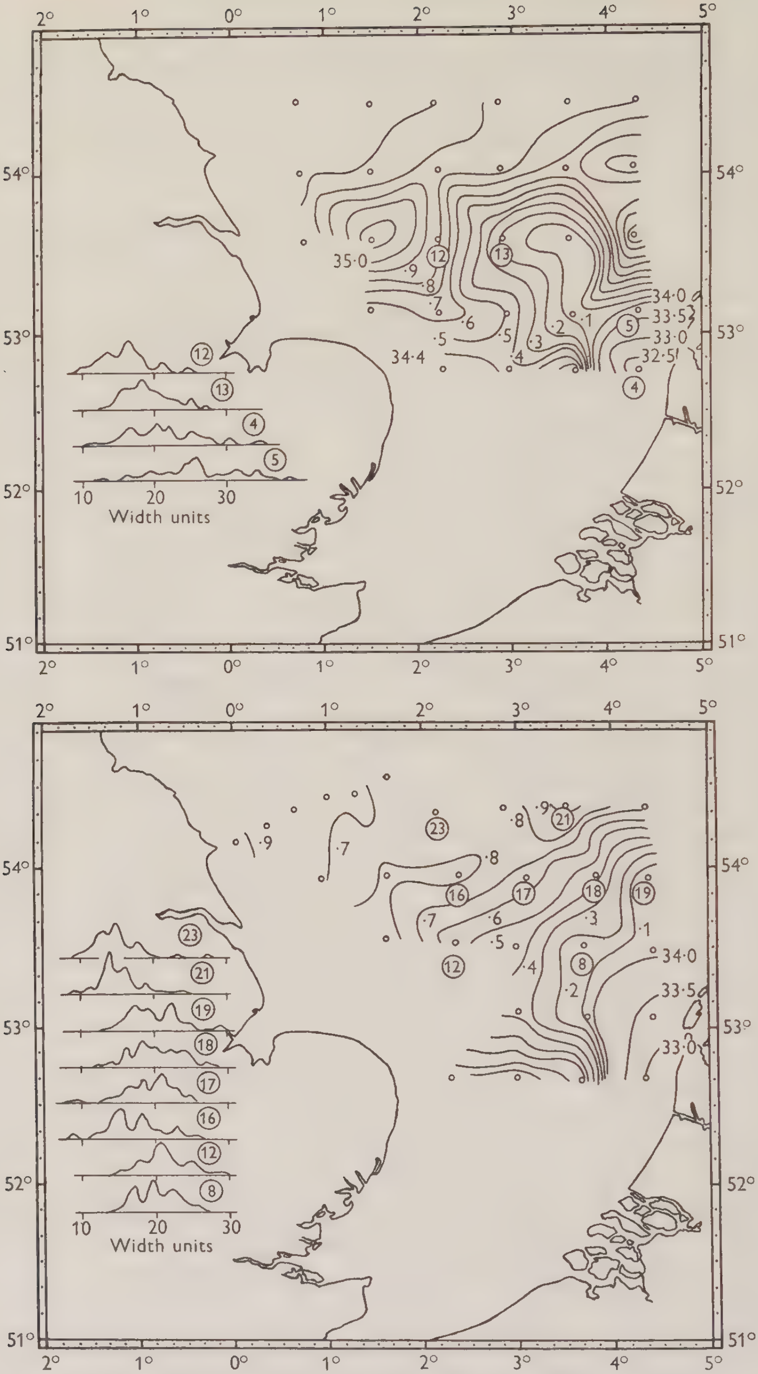


Fig. 1. *Biddulphia sinensis* width frequencies for various stations in the southern North Sea between 1932 and 1938. The ordinate is marked in 5s.


2°
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Width units

Fig. 2. Surface salinities on Cruise N, 1937 (above) and on Cruise O, 1937 (below) showing stations at which 100 width measurements of *Biddulphia sinensis* were made (ringed figures) and on the left the actual width frequencies from these stations.

positions from which samples were taken and the corresponding width distributions. The relation between surface salinity and cell width seems close in these cases, and even when all the fifty-two samples available over the period 1932–38 are taken together there is a negative correlation with the surface salinity of −0·43. This would happen by chance less than once in a hundred times. Similar correlations with density of population and with surface temperature (−0·06 and −0·17) were found to be of negligible significance.

Lucas & Stubbings (1948), using material from the Hardy continuous recorder in the southern North Sea between 1931 and 1938, measured samples amounting to 20,000 cells, most of them from 1938 material. From their distribution when compared with the data published in the *Bulletin Hydrographique* these workers inferred that the wider cells were found in the water of lower salinity mainly near the continental coast. They distinguish narrow, medium and wide cells in their samples, but on a line from Hull to Bremen in November 1938 they figure (p. 156) what at first appears to be a unimodal population changing abruptly near the Friesian Islands into a bimodal one with subsequent increasing emphasis on the larger mode. This latter appearing in Lucas and Stubbings's results in November seems to correspond with a similar one found by me at Station P 5 in October 1938, 20 miles off the Texel.

Consideration of the facts presented up to this point would make it appear that *B. sinensis* must adjust its width to changes of salinity fairly rapidly so that it would not be possible to follow and identify a population by its size distribution. On the contrary, it is rather more likely to be an index of contemporary salinity, and shows no steady diminution of size over a long period followed by a sudden return to maximum width as a result of the formation of auxospores as happens in *Rhizosolenia styliformis*.

However, when all the samples are grouped together by years (Fig. 3 and Table II), it is seen that there is a sharp rise in the chief mode of the size-frequencies between 1935 and 1936 which is reminiscent of that shown by the individual samples of *Rhizosolenia styliformis* taken in these years. There is also a slight rise in the mean size between 1932 and 1933 which, on account of the poor sampling in 1932, might have been thought of as of no significance if I had not been afforded the opportunity of examining some of Dr Lucas's unpublished measurements. With the latter's kind consent I have prepared Fig. 4 from these data. The figure shows the percentage frequency distribution of *Biddulphia sinensis* for the same general area as my material for the years 1932–38. It will be seen that, in addition to the increase in the modal frequency between 1935 and 1936 there are indications of increases from 1932 to 1933 and 1937 to 1938. It is as if, for *B. sinensis* as for *Rhizosolenia styliformis*, the same law of diminishing division followed by a sudden increase is being observed—if one adds together for each year all the separate samples which in some way appear adjusted in width to their contemporary salinity.

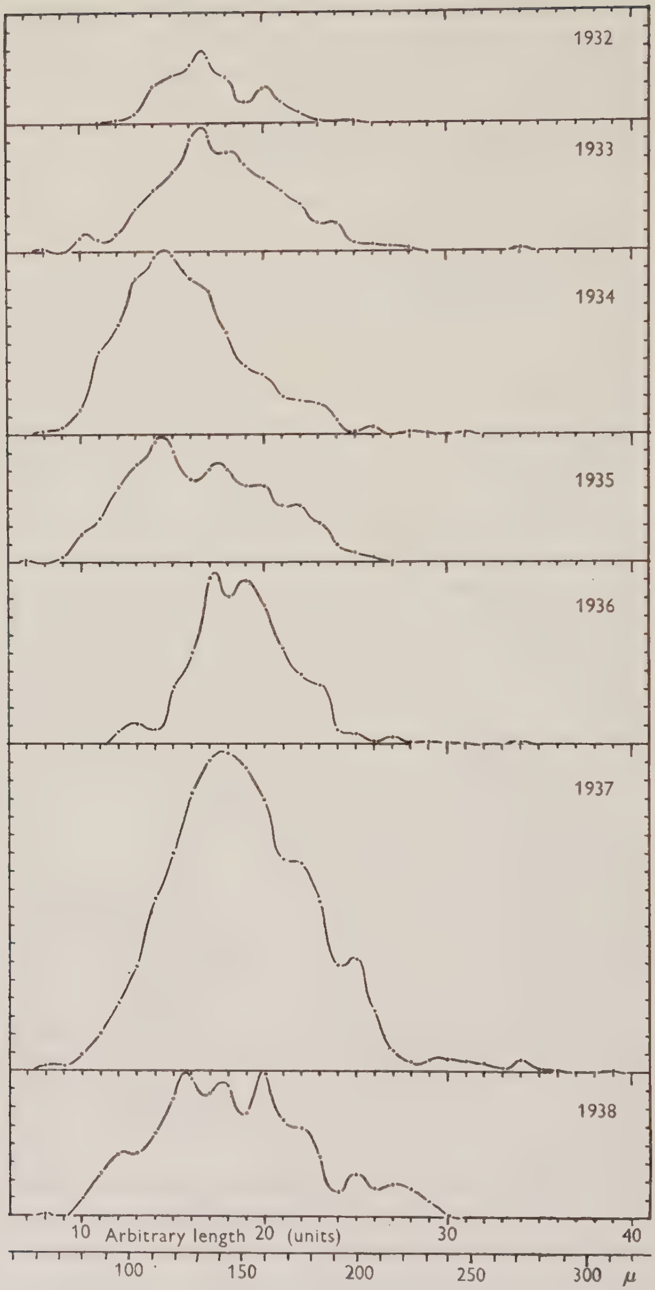

Fig. 3. *Biddulphia sinensis* width frequencies for the whole years 1932–38. The ordinate is marked in 10s.

TABLE II. MEASUREMENTS OF *BIDDULPHIA SINENSIS* DIAMETERS, FOR SEPARATE YEARS 1932–38

	Measurement groups (8 μ units)																																
Year	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	Mean
1932	—	—	—	—	2	5	9	25	28	30	35	22	10	23	3	6	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	18·2
1933	—	2	1	8	11	15	31	32	58	71	57	49	43	35	31	18	13	13	4	5	1	1	—	—	—	—	1	—	—	—	—	—	18·7
1934	—	5	7	25	48	82	91	104	88	82	60	55	38	22	18	22	11	3	3	2	1	2	—	1	—	—	—	—	—	—	—	—	16·7
1935	1	1	4	22	27	57	61	53	63	46	56	42	37	46	26	29	14	10	2	3	—	—	—	—	—	—	—	—	—	—	—	—	17·6
1936	—	—	—	—	3	10	8	18	40	77	77	97	89	58	49	32	21	7	2	2	5	2	1	—	1	—	1	—	—	—	—	—	19·9
1937	1	4	4	13	32	39	80	123	137	146	178	172	175	125	120	83	93	64	37	26	7	8	7	6	4	5	4	5	2	—	—	1	19·4
1938	—	1	6	18	25	38	41	48	73	72	71	66	79	61	47	44	20	17	21	17	15	14	6	—	—	—	—	—	—	—	—	—	19·4

TABLE III. GROWTH OF *BIDDULPHIA SINENSIS* CULTURES SHOWN BY CELL NUMBERS; AND MEAN SIZE OF CELLS

Cultures set up on 11 August 1950 in a north window. Size expressed as mean widths and lengths of samples of 100 cells taken on 15 and 21 August 1950, in units of 4 μ.

Date in August 1950	Sea water with erdschreiber	Sea water with erdschreiber diluted with distilled water of percentage		
		10	20	30
	Cell numbers			
12	6·75	5·0	4·75	6·5
13	18·75	37·25	22·75	20·75
14	53·5	95·25	80·0	73·5
15	117·5	178·25	153·0	162·75
16*	197·75	253·75	251·5	299·25
17	462·5	650·0	487·5	550·0
18	1,187·5	862·5	1,200.0	1,312·5
19	1,937·5	2,725·0	2,162·5	2,500·0
21*	5,262·5	7,462·5	8,312·5	6,725·0
22	12,500·0	9,375·0	25,625·0	18,125·0
24	37,500·0	51,250·0	54,375·0	63,125·0
26	96,875·0	96,250·0	101,875·0	119,375·0

	Cell shape							
	Width	Length	Width	Length	Width	Length	Width	Length
15	68·5	93·6	70·1	93·9	69·9	92·45	68·3	88·9
21	67·3	93·3	68·0	93·4	66·5	92·5	66·8	88·9

* Subcultured.

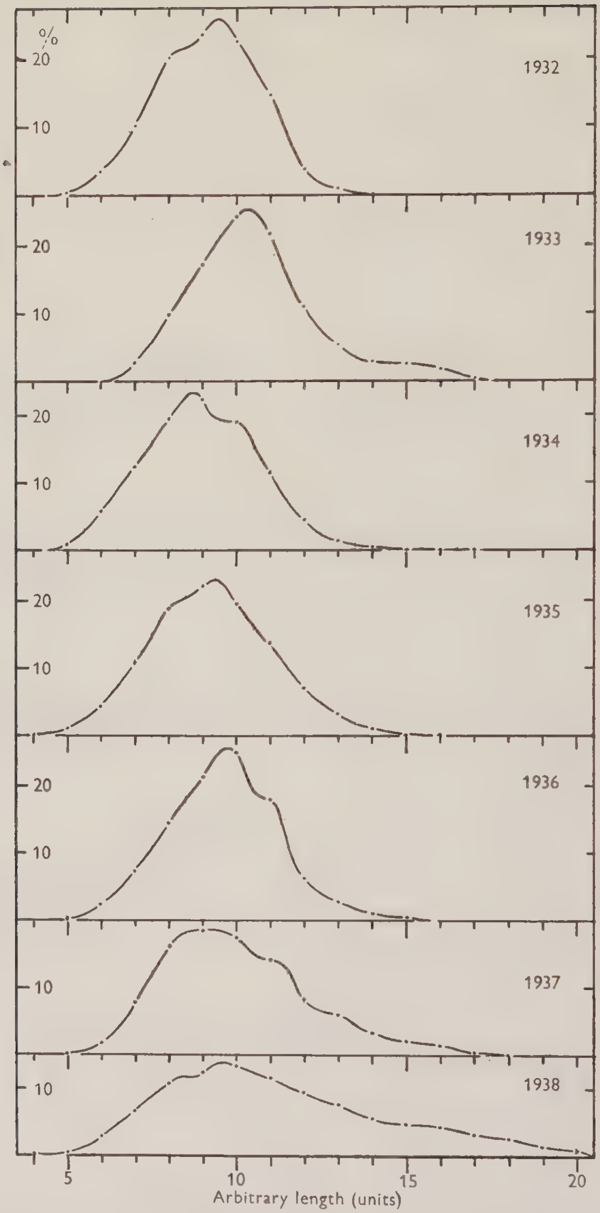

Fig. 4. *Biddulphia sinensis* width frequencies for the whole years 1932–38 in arbitrary units of approximately 12 μ. Lucas and Stubbings's material expressed as percentages along the ordinate.

Egusa (1949), who has worked on this species from a coastal bay in Japanese waters from September 1946 to April 1948, found a large and small diameter group alternating, the former dominating in winter and the latter in summer. No auxospore formation is described and there was no horizontal distribution of the samples which would indicate whether the individuals came from an identical stock. If the cells did in fact come from the same stock these observations suggest the annual formation of an auxospore generation in an environment where there is little change in the salinity.

FACTORS AFFECTING SIZE IN *BIDDULPHIA SINENSIS*

Width adjustment in diatoms is well known to occur downwards by division and upwards and suddenly by auxospore formation, and we may examine how this known method of diminishing growth and auxospore formation could produce the observed situation for *B. sinensis*. First, reference may be made to the work of Schreiber (1931), who figured and observed auxospore formation in *B. sinensis* in material collected off Heligoland on 19 July 1926, and at a time when the salinity—28·6‰—had reached a lower value than the preceding and following days and lower than the July average for 15 years—31·5‰. Schreiber also quotes Mielck as noting that *B. sinensis* is smaller in the northern than the southern North Sea.

In view of the observations on this species at Heligoland and the fact that he was able to produce abundant auxospore formation in *Melosira nummuloides* by lowering the salinity, Schreiber was of the opinion that the greater width in North Sea diatoms is due to more auxospore formation in an area of lower salinity. The correspondence of lower salinity with auxospore formation is also pointed out in Part I of this work (Wimpenny, 1936), where the distribution of *Rhizosolenia alata* is examined, and again in Part II (1946), where it was thought worth noting that a commencement in the lowering of salinities in 1935–36 corresponded with the origin of a new auxospore generation of *R. styliformis* in 1935 and its development in 1936. No auxospore formation has actually been seen in my material, but in a collection taken on the same voyage (Station 23, Cruise P, 1935) as that on which some of the present measurements were collected, what appeared to be newly formed auxospores and detached parent cells were seen. These are reproduced in Fig. 5. They occurred in the same year as did those of *R. styliformis* and, if we lump all the years' samples together, precede a year in which there was a sudden increase of size. This circumstantial evidence then favours the thesis of an auxospore formation in 1935 following populations which were on the whole diminishing in size and preceding a wider population which had also diminished in size by 1937. There is also the suggestion particularly supported by the width distributions communicated to me by Dr Lucas, that there may have been auxospore formation in 1932 or 1933 and possibly in

1938. Unfortunately the observations do not go beyond 1938 so that it cannot be said whether an unusually wide population occurring at Station 5 of Cruise P off the Dutch coast in 1938 could have been the precursor of a new auxospore generation.

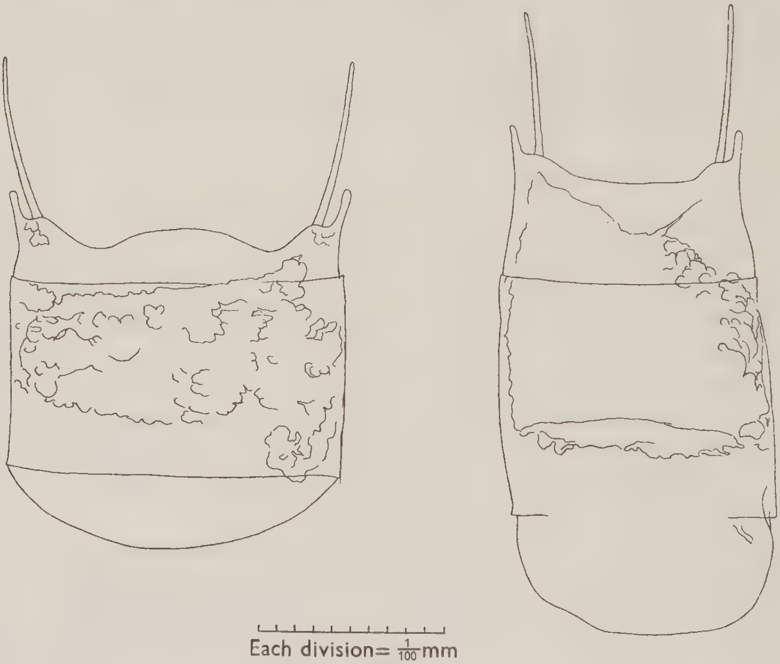

Fig. 5. Drawings of *Biddulphia sinensis* cells from station 23 of *George Bligh* Cruise P, 1935.

It is very puzzling to account for the big differences of widths in the same voyage or season, but three reasons occur to me as affording a possible mechanism. These are: (*a*) the frequent origin of auxospore generations in the less saline water; (*b*) direct and immediate adjustment of cell-size to the salinity of the surrounding water, perhaps by some osmotic action taking place at cell division; and (*c*) differential survival or growth of cells in water of different character, particularly with reference to changes of salinity.

In an attempt to test explanations (*b*) and (*c*), I made some simple experiments in 1950 on a culture of *B. sinensis* which Mr D. Jefferies of the Fisheries Laboratory, Lowestoft, had succeeded in establishing. Unfortunately, the cells of this culture were already similar in width to the wider fraction of those found in the sea, so that the fact that my first series of experiments in which the erdschreiber-enriched sea water was diluted to various degrees (Table III) showed no auxospores may only have meant that auxospore formation is not possible above a certain size. The dilution experiments also showed little or no difference in growth rate over the period of 21 days (about a division a day for most of the time). However, for the first two days it did appear that dilution accelerated the rate of growth, 10% being the most effective and 30% the least. There were no increases in width attributable to osmotic swelling or any other cause. There also appeared to be little or no mortality in this series and the diminishing cell widths suggested that on the average the cell wall might be expected to be about 1·5 μ thick. These experiments were carried out in a room with a north light where the day temperature varied between 18° and 20° C.

TABLE IV. GROWTH OF *BIDDULPHIA SINENSIS*, IN CELL NUMBERS

Cultures set up on 23. viii. 50

Date	In erdschreiber-enriched seawater at 24° C	In erdschreiber-enriched seawater at 10° C
23. viii.	11	15
24. viii.	20	16
25. viii.	27	22
26. viii.	33	38
28. viii.	40	42
29. viii.	42	54
30. viii.	55	62
31. viii.	80	64
1. ix.	78	72
2. ix.	75	92
4. ix.	90	103
5. ix.	—	93
6. ix.	—	85
7. ix.	92	90
8. ix.	91	96
9. ix.	93	101
11. ix.	98	108
12. ix.	95	98
13. ix.	98	101
14. ix.	100	100

TABLE V. LENGTH AND WIDTH OF *BIDDULPHIA SINENSIS* CELLS AT DIFFERENT TEMPERATURES

Measurements in 4 μ units. Light supply 550 Lux

	Parent population	Population at 24° C			Population at 10° C		
	23. viii.	29. viii.	2. ix.	12. ix.	29. viii.	2. ix.	12. ix.
Observations	100	29	50	41	27	46	33
Mean width	66·4	62·0	60·5	62·4	61·2	61·0	56·9
Mean Length	106·2	93·0	97·8	121·4	112	110·9	134·1

In the second series two experiments run at 24° and 10° C (Tables IV and V) showed little difference in growth, which stopped after three divisions, but many morbid cells appeared, more at the higher temperature. Cell measurements indicated that the mortality was selective, the higher temperature culture being left with cells that were wider and shorter than those grown at the lower temperature.

As far as they go, therefore, these experiments do not suggest that immediate swelling or formation of auxospores follows a lowering of salinity where the cells are of the larger size. On the other hand they do suggest that an environmental difference, in this case temperature, can act on cell shape through selective mortality at different values.

SUMMARY

The cell widths of samples of *Biddulphia sinensis* taken from Hensen net hauls made in the southern North Sea between 1932 and 1938 correspond rather closely to the contemporary salinity samples, but not to the density of population or to temperature.

The width distributions of *B. sinensis* do not give a persistent and characteristic guide or mark to populations of this species in the way that is characteristic of *Rhizosolenia styliformis*, but rather give a rough indication of the salinity of the water in which they are found.

There is nevertheless some circumstantial evidence pointing to a 3-year auxospore cycle similar to that shown by *R. styliformis* in the same area. It is, however, much masked and overlaid by other causes of change in cell width.

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STUDIES ON MARINE FLAGELLATES

III. THREE FURTHER SPECIES OF *CHRYSOCHROMULINA*

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(With total of 76 Figures in text and on Plates I–IX)

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Introduction

Two of the new species of *Chrysochromulina* to be described here are somewhat more like the type species (*C. parva* Lackey) than were any of those included in our last communication (Part II of this series—Parke, Manton & Clarke, 1955). The diagnostic generic character of the presence of three filiform appendages arising close together is shared by all, and as before two of the appendages are flagella of equal or almost equal length and the third is a special organ to which the name *haptonema* has been given (Gr. ἅπτω to attach, νῆμα pl. νήματα, a thread) (1955, p. 581). In our previous species the haptonemata when fully extended were relatively short, being not very different in length from the flagella. When not extended it was coiled in a flat spiral. Our three new species all have much longer haptonemata, of the order of twice the length of the flagella in *C. ericina* and three to five times the length of the flagella in *C. ephippium* and *C. alifera*. In all three species the haptonema is distinctly thinner than the flagella, a character probably connected with the absence of a conspicuous sheath (contrast with *C. kappa* and *C. minor* of our previous paper) and when not extended it is coiled in a solenoid. Several of these characters agree fairly closely with those given by Lackey for *C. parva*, as will be explained in connexion with *C. ephippium* (p. 406), though the comparison cannot yet be made in full detail since *C. parva* is an American freshwater species which has only been found once (Lackey, 1939) and it has not yet been available to us for study.

Our principal comparisons are therefore with *C. kappa*, *C. minor* and *C. brevifilum* of our previous paper, and here there is marked agreement in the main features of structure and life-history. As before we are dealing with pigmented flagellates showing many of the metabolic characteristics of the photosynthetic Chrysophyceae combined with the habit of phagotrophic feeding. Ingestion of graphite has been demonstrated with the light microscope in all three new organisms and evidence is included in the plates. As before the body surface is apparently covered by translucent scales without mineral impregnation, which take up cresyl blue. Their details can only be accurately seen with the electron microscope but they provide important criteria for the specific diagnoses. The very long spines of *C. ericina* can, however, with difficulty be seen individually with the light microscope.

Though we are still ignorant of the functional significance and mode of formation of the scales, our three new species all suggest that they may contain two layers of material. All the spineless scales show a characteristic and different pattern on their two surfaces, and since similar patterns occur on the flat parts of the spined scales (with special clarity in *C. ephippium*) we have been able to identify the outer and inner surfaces respectively. These differences were not detectable in our previous species.

Another detail of difference from our previous species, the significance of which cannot yet be wholly evaluated, is that the point of emergence of the three appendages is somewhat asymmetrical in relation to the rest of the cell. In *C. ericina* the point of emergence is no more than slightly 'off centre' and the two flagella seem isodynamic in function as well as equal in length. In the other two species the point of emergence is more definitely ventral under certain conditions, though the capacity for the body to change its shape hampers exact description. We have not been able to detect in these any marked or constant difference in either the length or the structure of the two flagella, but when the organisms are studied alive the flagella are definitely heterodynamic in behaviour. The significance, if any, of this observation will be discussed on a later occasion, but it is perhaps of importance to draw attention to the facts as described for *C. ephippium* (pp. 403–5) and *C. alifera* (p. 412) and to the preliminary discussion on p. 413.

With regard to life history, an advance on our previous work has been the more detailed observation of the emergence of cells of the motile phase from the dormant, walled, cells of the non-motile phase in *C. ephippium* (pp. 405, 406). In all our other species we have described empty membranes with a circular opening as providing strong suggestive evidence that such a transition takes place and the new observations demonstrate the truth of this supposition.

In presenting the facts for individual species we have necessarily had to do so in considerable detail, since it cannot yet be known with certainty which characters are limited to, and therefore diagnostic of, single species, and which will be found common to assemblages of species when more are known. We have already evidence that the remarkable spines of *C. ericina*, for example, are not an isolated phenomenon but that they will recur in various ways in the specific descriptions of other organisms which have not yet been fully studied. We have therefore placed on record everything that we have been able to ascertain about the species to be described, as studied in unialgal cultures, apart from certain fundamental characters common to all species of *Chrysochromulina* that have already been discussed (1955).

Our methods of study have been exactly as enumerated before (1955, pp. 281–3).

As before, our grateful thanks are due to Dr J. E. Morton, of Queen Mary College, for translating our specific diagnoses into Latin, and to Miss D. Ballantine for testing these organisms for their possible toxicity to fish.

SPECIFIC DESCRIPTIONS

Chrysochromulina ericina n.sp., Parke & Manton

(Lat. *Ericinus*—like a hedgehog)

Diagnosis

Motile cells usually ovoidal to nearly oblong, very slightly flattened in one plane, showing marked metaboly, 6–10 (exceptionally 5–12) μ in length, 5–8 (exceptionally 4–10) μ in breadth; flagellar pole obliquely truncated with a slight, almost central depression. Two flagella and one haptonema arising close together not quite centrally from the depression: the flagella equal, homodynamic, 2–2½ times body length, smooth, gradually attenuated to a small knob (E. M. observation); the haptonema thinner than the flagella, 4–5 times body length when fully extended with a small basal swelling, a club-shaped tip but no clearly marked translucent sheath obvious under the electron microscope. The periplast of a pectic nature covered by very thin transparent, sculptured, dimorphic scales, details visible only under the electron microscope. Scales without spines very numerous, $0{\cdot}5 \times 0{\cdot}6\,\mu$ to $0{\cdot}7 \times 0{\cdot}9\,\mu$, with a pattern of radiating ridges on one side and a slightly raised rim surrounding irregular crossed striations on the other. Spined scales, 28–30 in number, the spine abruptly truncated and slightly tapering, 9–12 (exceptionally 15) μ long, 0·2–0·3 μ wide, arising from a circular (or conical?) base, 1–1·4 μ wide, marked with concentric striations on its outer side.

Cells uninucleate, no stigma. Chromatophores usually 2 or 4, sometimes 6 or 8, deep golden brown; in motile phase saucer-shaped, ellipsoid, or oblong, frequently bifid towards non-flagellar pole, parietal, with a single globular body (pyrenoid?) on inner face placed near margin towards the non-flagellar pole; in non-motile phase deeply lobed or stellate. Oil and leucosin produced. Ejectile muciferous bodies generally distributed in peripheral cytoplasm. Nutrition phototrophic and/or phagotrophic. Non-toxic to fish.

In motile phase asexual reproduction by fission into 2 daughter-cells of equal or unequal size; in non-motile phase reproduction (asexual?) by successive fission of amoeboid cells to produce 4 ovate daughter-cells with walls, walls faintly brownish and slightly rugose on the exterior; motile phase probably liberated from walled daughter-cells through a pore.

Habitat: the sea at position (Plymouth Laboratory Station L4) Lat. N. 50° 15′, Long. W. 4° 13′ (15 May 1949, type culture) at surface; and at position (International Station E1) Lat. N. 50° 02′, Long. W. 04° 22′ (13 July 1955) at 20 m. Type culture (Plymouth no. 25) deposited with the Type Culture Collection, Cambridge; preserved material and photographs lodged with the Marine Biological Association, Plymouth, England.

Cellula motili, generaliter ovoidali aut ferme oblongio, paululum planato in uno aspectu, formam conspicue mutanti, longitudine 6–10 μ (rare 5–12 μ), latitudine 5–8 μ, rare 4–10 μ; apice quo inserta flagella oblique truncato, leviter depresso prope centrum. Duobus flagellis et unico haptonemate conjunctim exorientibus e depressione; flagellis aequis, homodynamicis, longioribus 2 ad 2½ quam cellula, teretibus, paulatim attenuatis et acutis; haptonemate teneriore quam flagellis, longiore 4–5 quam cellula, cum maxime extensus, leviter tumescenti prope originem, clavato extremitate sed nulla tunica externa semidiaphana ut videtur per microscopiam electronicam. Periplasto, pectico natura, induto delicatissimis sculptis squamis diaphanis, sculptura invisibili nisi per microscopiam electronicam, manifestis sub duobus formis: altera forma, sine spinulis, pernumerosis, longis 0·6–0·9 μ, latis 0·5–0·7 μ, ornatis radiantibus striis ad inferiorem aspectum, margine paululum elevata circumdanti irregulares decussatas strias ad superiorem aspectum: altera forma, squamis 28–30 numerosis, spinulis praeditis, quoque spinulo subfastigiato sed et abrupte truncato ad extremum, 9–12 μ (rare 15 μ) longo, 0·2–0·3 μ lato, exoriente e basi circulari aut conica, ornata concentricis striis ad aspectum superiorem.

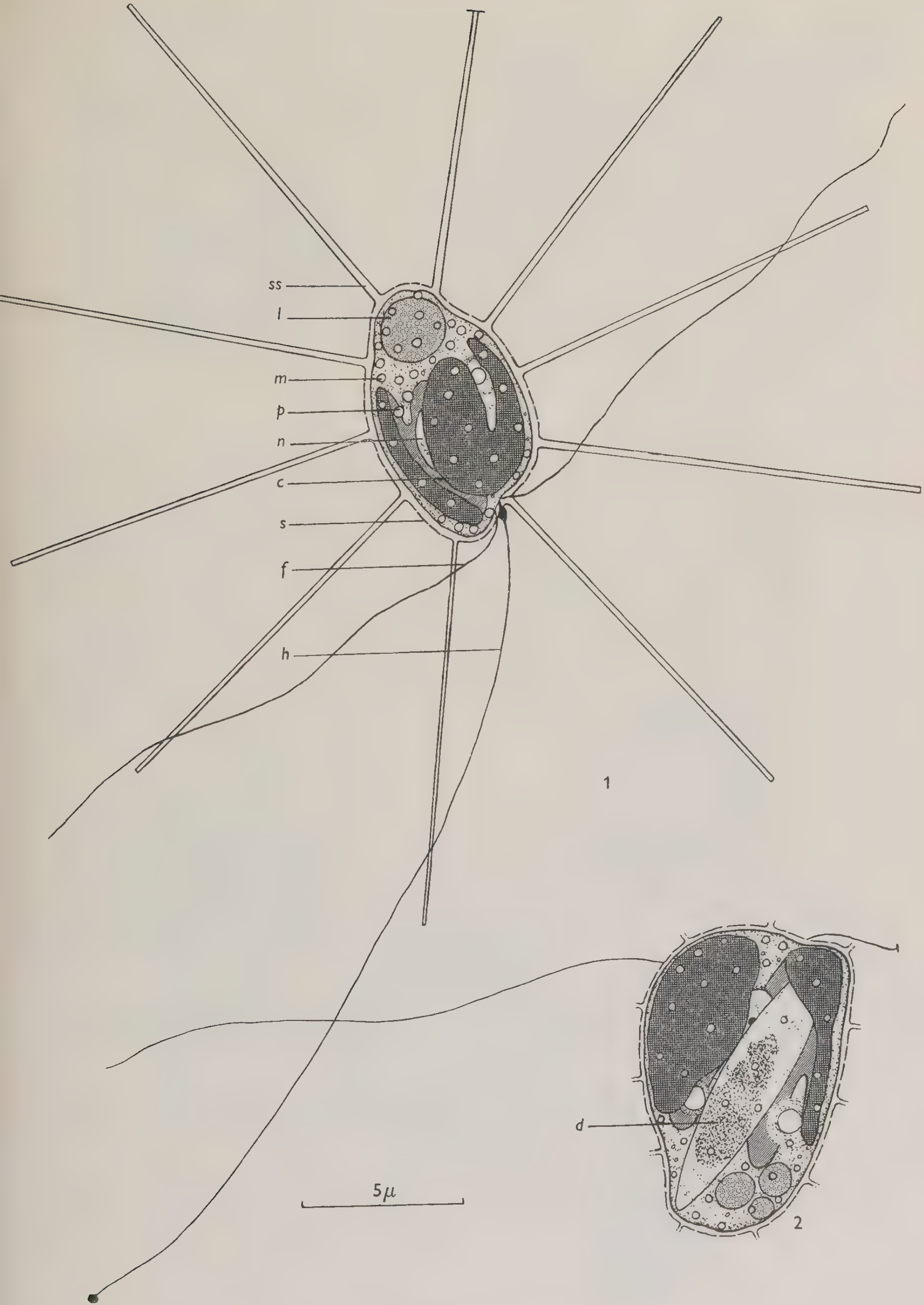
Nucleo unico, nullo stigmate, chromatophoris ex norma 2 aut 4, nonnunquam 6 aut 8, profunde aureo-brunneis; in statu motili cellulae crateriformibus, ellipsoidalibus aut oblongis, saepe bifidis versus apicem cellulae quo desunt flagella, parietalibus;

Legends to Text-figs. 1–2

Chrysochromulina ericina n.sp. (× 5000)

Fig. 1. Individual with two dividing chromatophores anchored by haptonema which is partly extended; the flagella are in the characteristic position adopted when the species is stationary. *c*, chromatophore; *f*, flagellum; *h*, haptonema; *l*, leucosin vesicle; *m*, muciferous body; *n*, nucleus; *p*, pyrenoid-like body; *s*, scale; *ss*, spined scale.

Fig. 2. Individual anchored by coiled haptonema which is hidden below protruding lobe of flagellar pole; cell contains an ingested *Navicula salinicola* Hust. (*d*).


ss
l
m
p
n
c
s
f
h
1
d
2
5μ

Text-figs. 1–2

unico corpore globulari (? pyrenoidali) in concavo aspectu prope marginem chromatophori posito versus apicem quo desunt flagella; in statu non-motili cellulae chromatophoris profunde lobatis vel stellatis. Cellula oleum leucosinumque parienti; corporibus muciferosis et ejectilibus undique distributis in cytoplasmate superficiali. Nutritione phototrophica necnon phagotrophica. Non toxica piscibus.

Generanti asexualiter in statu motili per fissionem in duas cellulas filiolas vel aequas vel inaequas magnitudine; in statu non-motili generanti (?asexualiter) per fissiones subsequentes cellularum amoeboidalium ad 4 cellulas filiolas ovoidales producendas, parietibus leve brunneis et paulum rugosis externe; maxime potest ut cellulae in statu motili ex cellulis pariete praeditis per foramen liberentur.

Habitat mare prope Plymouth ad locationem Lat. N. 50° 15′, Long. W. 4° 13′ (15 Mai 1949—cultura typica) ad summum mare; necnon ad locationem Lat. N. 50° 2′, Long. W. 4° 22′ (13 Jul. 1955).

Description

The form range of the motile cells is illustrated in Figs. 1–5 and in the photographs of Pl. I. The slight flattening of the body can be most easily observed when looking down on the non-flagellar pole (Fig. 3) and the pronounced metaboly is most obvious when individuals are ingesting or have ingested cells of other species (Figs. 2, 4, 5). In an actively growing culture 85% of the cells are from 6 to 10 μ in length, while 5% are between 5·0 and 6·0 μ. The remaining 10% are incipient fission stages from 10 to 12 μ in length.

The flagella and haptonema arise close together slightly to one side of a shallow depression at the obliquely truncated pole (Fig. 13, Pl. I; Figs. 1, 4, 5). The flagella (Figs. 14, 15, Pl. II) are very thin as in *C. kappa* Parke & Manton,

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Legends to Text-figs. 3–9

Chrysochromulina ericina n.sp. (× 5000)

Fig. 3. Characteristic position of an anchored cell when the haptonema is not extended; view is looking down on non-flagellar pole with flagella lying straight out below body; bacteria (*b*) in vacuole adjacent to pyrenoid-like body.

Fig. 4. Individual with four chromatophores swimming with flagella and haptonema behind body in the position characteristic for the species during rapid swimming; muciferous organelles exuding contents; recently ingested *Chlorella stigmatophora* cell (*i*) at non-flagellar pole and an empty wall of a *Chlorella* cell immediately after ejection from the end of a colourless tube.

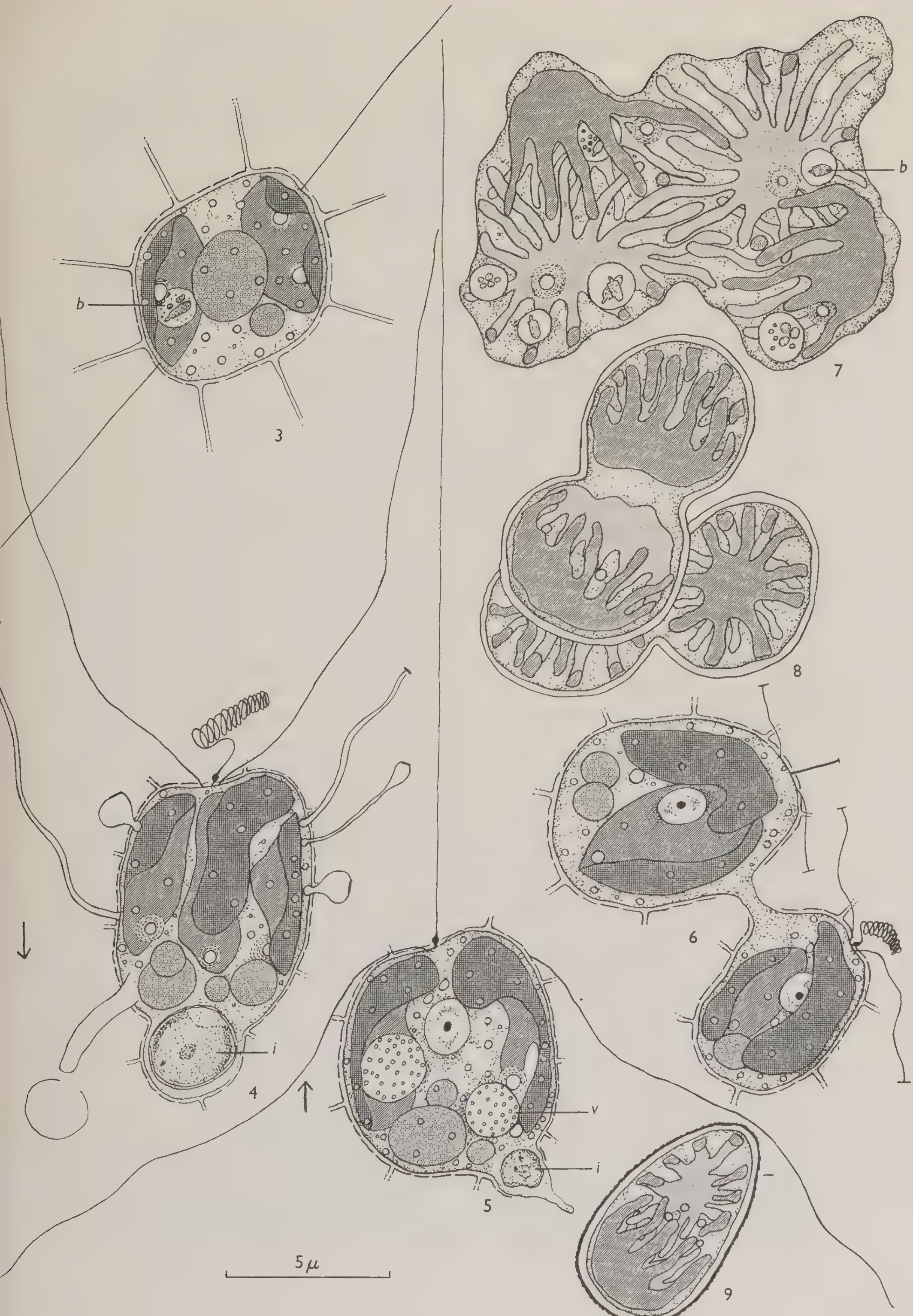
Fig. 5. Individual swimming with flagella and haptonema in front of the body in characteristic position, haptonema fully extended; a cell of *Oicomonas* (*i*) being ingested and large vacuoles (*v*) containing granules showing Brownian movement adjacent to both pyrenoid-like bodies.

Fig. 6. Late fission stage just before separation of daughter-cells.

Fig. 7. Large amoeboid individual with four deeply lobed, pale chromatophores and four pyrenoid-like bodies surrounded by non-refringent material; ingested bacteria (*b*) dancing in vacuoles.

Fig. 8. Second fission of a large walled cell almost completed, to give four small, walled daughter-cells.

Fig. 9. Small rugose walled daughter-cell with two stellate chromatophores and two pyrenoid-like bodies.


b
3
b
7
8
6
4
i
5
v
i
9
5 μ

Text-figs. 3–9

and difficult to see under the light field, so also is the haptonema which when fully extended is much longer than in the species previously described (approximately twice the length of the flagella, Pl. I; Fig. 5).

Like *C. kappa* the haptonema has a club-shaped tip (Pl. II) and a swollen base (Fig. 13, Pl. I; Pl. II; Fig. 17, Pl. III) but the base is smaller and more ovoid than in *C. kappa*; the delicate sheath of the haptonema is very inconspicuous (Fig. 16, Pl. II).

The two types of scales may be seen at low magnifications in Pls. I and II and in greater detail in Pls. III and IV. While the plate-scales can only be separately detected with the electron microscope, the spines are sufficiently large to be visible and even counted with the light microscope if the cells are dried. They are just detectable in Figs. 10 and 11, Pl. I, and they become even clearer if methylene iodide saturated with sulphur at 30° C. is added to dry preparations (Hollande, 1952, p. 472). They are too translucent to be visible without staining on living or undried cells, but their presence is sometimes detectable after addition of graphite to a culture by the adhesion of small masses of graphite to their tips. By this means their length and distribution on the living cell can be assessed and we believe them to be uniformly distributed on the body surface with their bases separated by a distance equivalent to the diameter of 2 or 3 plate-scales. They are very readily displaced after death, and especially by the act of drying.

The chromatophores of the motile phase are parietal and deeply pigmented and frequently appear striated, their shape and position changing with the metaboly of the body. They tend to lie towards the flagellar pole (Figs. 1–6). In the non-motile phase they are paler and deeply lobed (Figs. 7–9). Individuals lacking chromatophores are normally not seen in cultures of this species except rarely after treatment with penicillin and streptomycin.

As in our previous species there is a refringent body, the so-called pyrenoid, present on the inner face of each chromatophore. Its position is eccentric, towards the non-flagellar pole (Figs. 1–6) as in *C. brevifilum* Parke & Manton, but it changes with the metaboly of the body. These pyrenoid-like bodies, 0·5 to 1·0 μ in diameter, appear greenish and are fairly conspicuous in some individuals but in others they are hardly visible; after osmic fixation they show up more clearly. Frequently these bodies are surrounded by non-refringent material, sometimes by quite large masses up to 2 μ across. The nucleus, placed nearly centrally in the body, is of medium size and can sometimes be seen in the living cells. Leucosin vesicles of various sizes, sometimes as large as 3 μ in diameter, are present, usually lying in the body towards the non-flagellar pole; small oil globules are distributed through the cytoplasm.

The muciferous organelles are quite large and appear to be generally distributed in the peripheral cytoplasm of the cell but with the metaboly of the body they can sometimes be seen in rows. When they expel their contents rapidly, straight threads up to 90 μ long can be shot out, but when they discharge slowly, as for instance when kept at a temperature of 22–24° C or when certain other organisms have been added to the culture, then the contents exude either as small balloons or as thin waving threads (Fig. 4) frequently showing what appears to be a small flat colourless disk sticking to them (Fig. 4). When extremely dilute cresyl blue is added to the living cells one sees almost immediately what appear to be minute disks shot out from the cell surface, which dance about for a time and then disappear. It could not be ascertained whether these disks were caps covering the organelles, as in Hovasse's discobolocysts (Hovasse, 1949), or surface scales pushed off during the discharge, but their capacity to stain a deep blue with cresyl blue suggests that they are probably caps, not scales, as the latter usually stain a pale violet colour. Immediately after the liberation of disks the contents of some of the organelles are discharged, some as small balloons, others as fine threads (Fig. 4).

Some distinct granules, possibly mitochondria, occur generally distributed in the peripheral region of the cell. They stain an intense blue with cresyl blue and under oil immersion can be seen to be connected together by a very fine blue network.

This species moves comparatively slowly with a fairly even rotation and little gyration. There is a marked phototactic reaction in spite of the absence of a stigma. As in the three species already described, swimming is most rapid when the flagella and haptonema are directed backwards and the haptonema is tightly coiled (Fig. 14, Pl. II; Fig. 4).

The rate of movement decreases with an increase of temperature up to 22–24° C. when movement becomes extremely slow, the individual then generally swimming with the haptonema in front of the body.

When not tightly coiled, the haptonema may be fully extended (Fig. 5), in front of, or behind the body, or only partly extended, the remainder appearing as a blob at the distal end (Fig. 11, Pl. I). When individuals swim with the haptonema foremost the flagella are held as in Fig. 5, but when they swim with the haptonema behind the body the flagella are held as in Fig. 4 with their free ends farther apart than the width of the body. The cells do not swim for long in one direction. They can stop suddenly by bringing the flagella to the position shown in Fig. 1, or they can suddenly change and move in the opposite direction by a flick of the flagella from the position of Fig. 5 to the position of Fig. 4 and vice versa. When moving with the haptonema extended in front of the body (Fig. 5) cells are frequently seen to jump back suddenly as if the haptonema had touched something obnoxious. The haptonema can also be bent over from side to side.

Quite long periods of anchorage are common, but the tip of the haptonema can seldom actually be seen attached to a surface, the cell body usually lying over it. The most characteristic position adopted by attached cells is shown in Fig. 3; the haptonema is tightly coiled below the body while the flagella appear as a straight line on either side. The flagella can either remain quite still or vibrate slowly, causing the body to show a slight dancing movement. Individuals can frequently be seen in the act of attaching with the haptonema nearly fully extended (Fig. 1). The haptonema may remain fully extended or it may coil up drawing the cell down to the surface of attachment (Fig. 2). In the latter position the haptonema becomes hidden owing to the asymmetry of the body. When the distal end of an attached haptonema can be seen, it is sometimes coiled in a flat spiral appearing as a disk with the point of attachment in the centre (Fig. 1). In other cases the haptonema can appear very short (and thicker?), as in *Prymnesium* species, without a visible disk at the attached end (Fig. 6). In these it is perhaps only the distal tip of the haptonema that is extended, the remainder lying coiled and hidden below the body since we have no evidence suggesting that the haptonema can actually contract, as opposed to coiling.

Phagotrophy is of common occurrence, the individuals ingesting bacteria and other organisms, usually up to a size of $3\,\mu$ but occasionally larger, the maximum ingested size observed being a diatom cell of $9 \times 3\,\mu$. In addition to graphite (Fig. 12, Pl. I), a number of cultures of different sized organisms were also used with the following results: ingestion of *Oicomonas* sp. $1\text{–}2\,\mu$

Explanation of Plates I–IV

Chrysochromulina ericina n.sp.

I

Fig. 10. A cell killed with osmic vapour and dried on a glass slide, with some detached scales marked by arrows. Photographed dry without a coverslip. Magnification × 1000.

Fig. 11. Two cells of the same.

Fig. 12. Three cells killed with osmic vapour after graphite feeding and photographed in a liquid mount with oil-immersion lens and visual light. Magnification × 2000.

Fig. 13. A low-power view of a cell dried and shadowed after vapour killing, on a formvar film, seen with the electron microscope. Micrograph M. 128·1, magnification × 3000.

II

Fig. 14. A cell showing flagella and a coiled haptonema. Electron micrograph M. 239·26, magnification × 5000.

Fig. 15. Tip of left-hand flagellum of Fig. 14, magnification × 10,000.

Fig. 16. A haptonema from the cell of Fig. 13 more highly magnified. Electron micrograph M. 128·2, magnification × 5000.

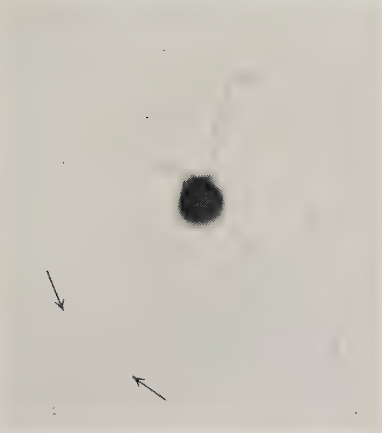
III

Fig. 17. The body of the cell of Fig. 13 more highly magnified to show scales and the bases of spines. Electron micrograph M. 128·3, magnification × 10,000.

Fig. 18. A group of detached spines and scales. Electron micrograph M. 128·9, magnification × 5000.

IV

Fig. 19. Part of Fig. 18 more highly magnified to show details of the scales and bases of the spines. Electron micrograph M. 128·13, 40 kV, magnification × 20,000.



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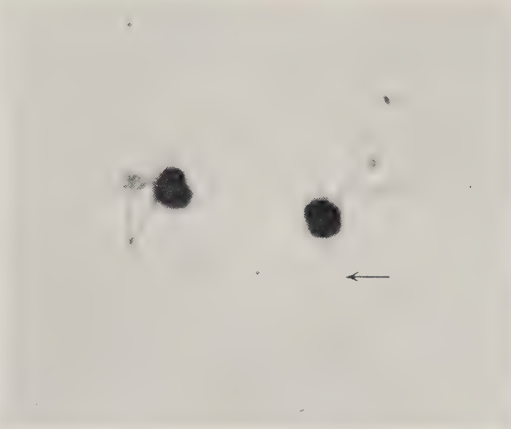

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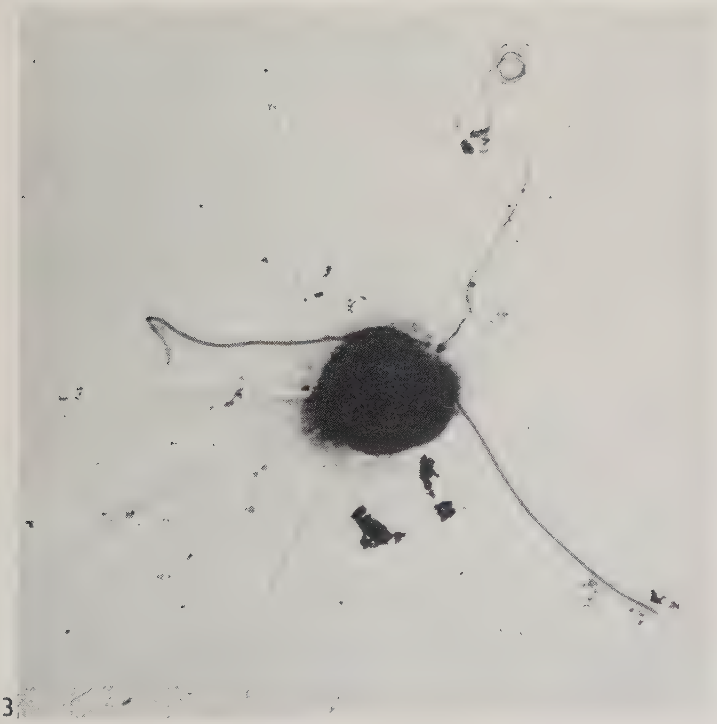

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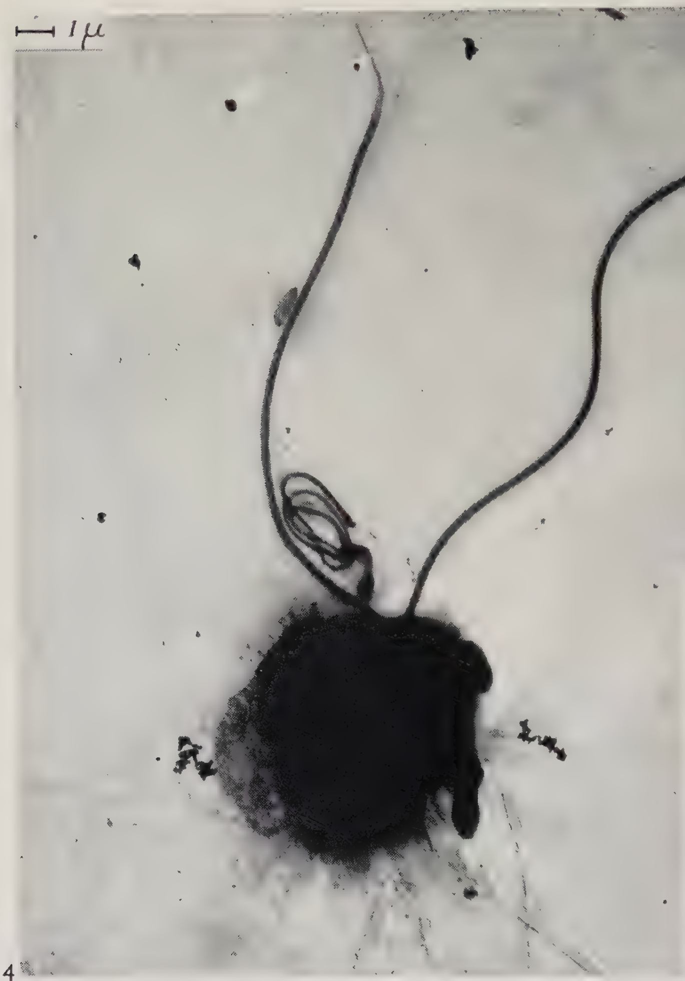
(*Facing p.* 396)



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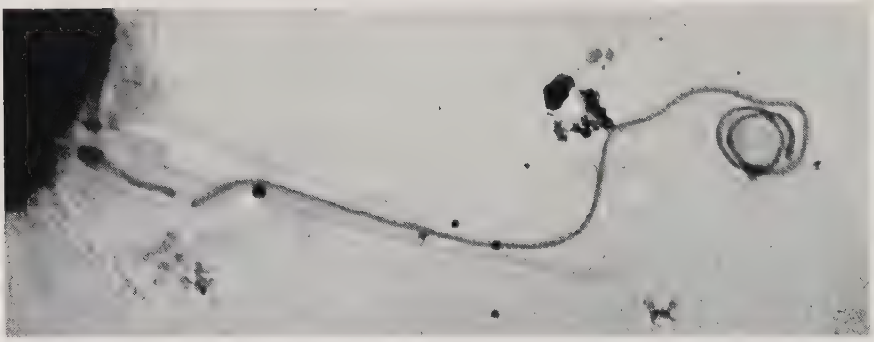

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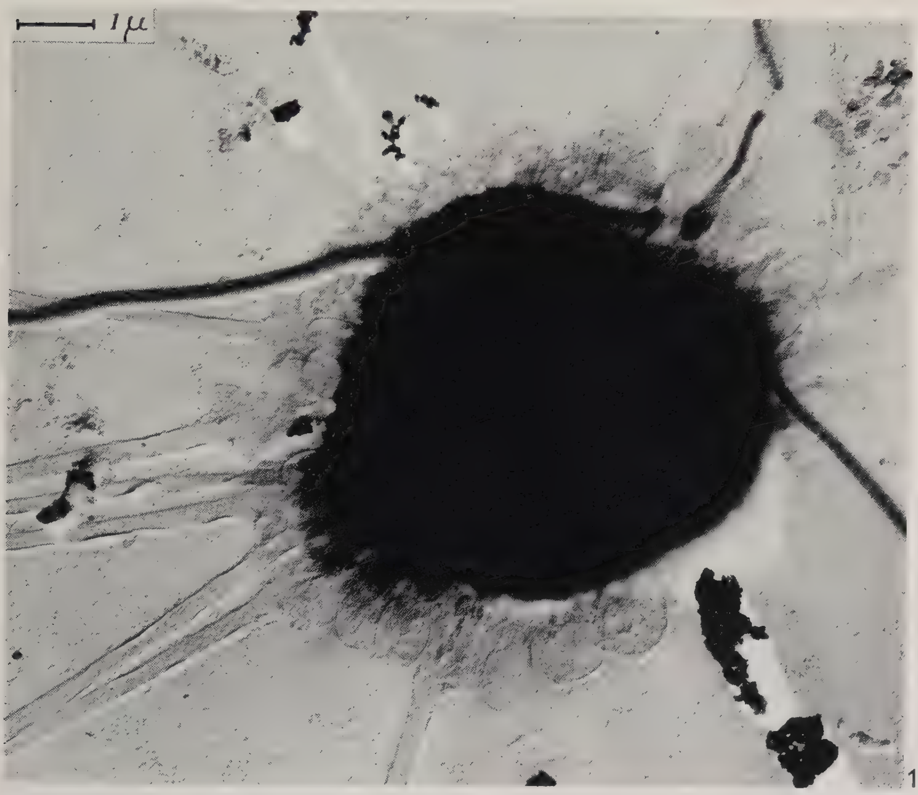

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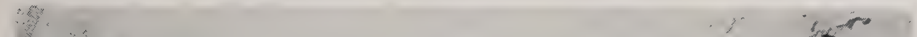

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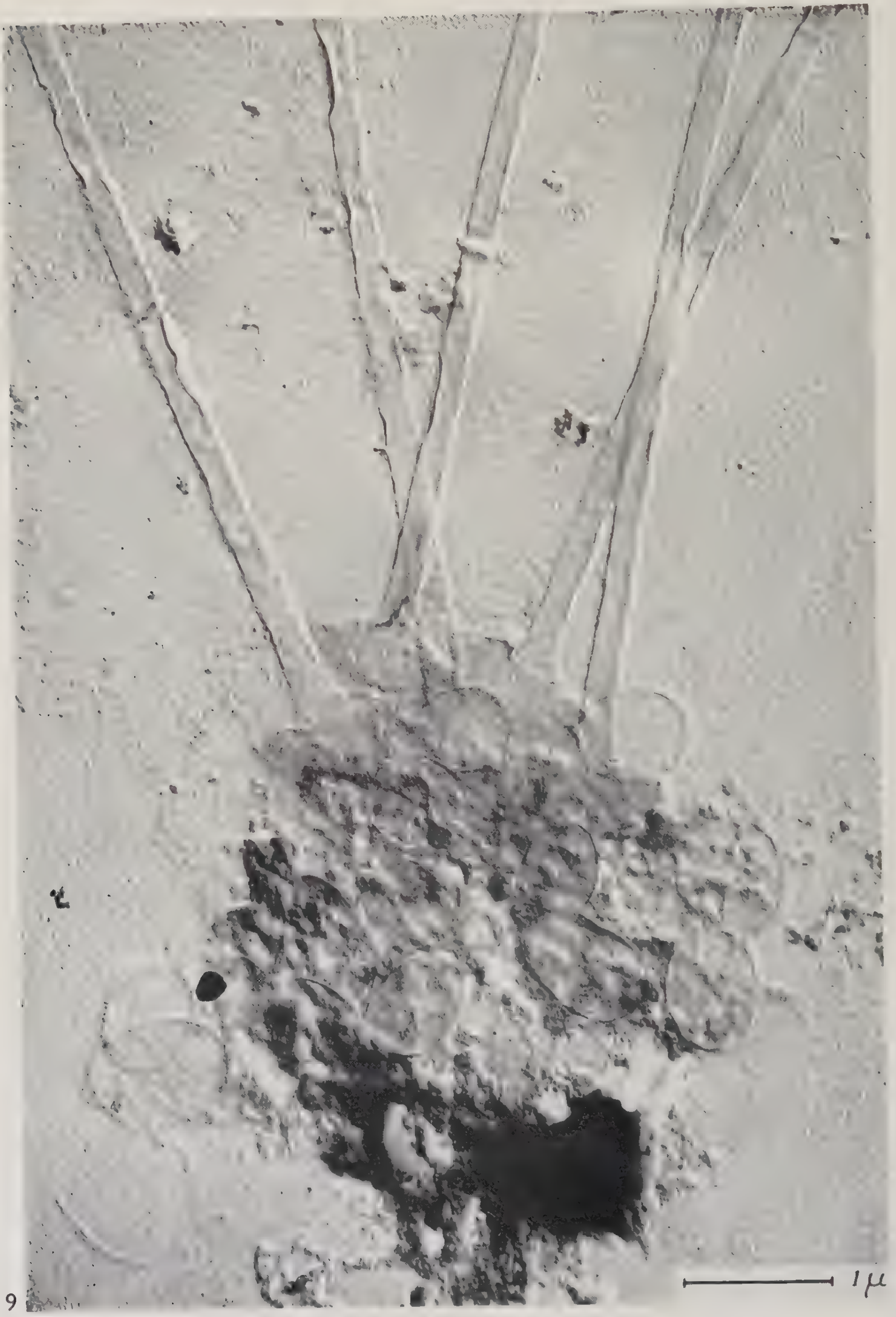

(Fig. 5), *Stichococcus cylindricus* Butcher 3–5 × 2 μ, Plymouth no. 55 (possibly a *Stichochrysis* sp.) 3–10 × 3 μ, and *Chlorella stigmatophora* Butcher 2·5–4·5 μ (Fig. 4) was fairly frequent; ingestion of the smaller individuals of *Nitzschia gotlandica* A. Cleve-Euler 6–10 μ L. and *Porphyridium cruentum* (Ag.) Näg. 4–12 μ diam. was not uncommon, but the ingestion of *Navicula salinicola* Hust. 9–10·5 μ L. was seen only once (Fig. 2) when a cell 9 × 3 μ had been ingested by an individual 10 × 6 μ. The following species were tested for ingestion with negative results: two *Dunaliella* spp. 6–12 μ, *Phaeodactylum tricornutum* Bohlin 8–35 μ and *Nannochloris atomus* Butcher 2–3 μ. One of the *Dunaliella* sp. (Plymouth no. 81) and the *Nannochloris* appeared to have an adverse effect on the *Chrysochromulina ericina*, whilst *Chrysochromulina* cells which had actually ingested *Chlorella* cells were believed to disintegrate afterwards, but the evidence is not yet absolutely conclusive. With the addition of certain unialgal cultures (e.g. *Dunaliella*, *Chlorella*, *Phaeodactylum*, *Navicula*) to the *Chrysochromulina*, the muciferous organelles of the *Chrysochromulina* cells were seen to exude their contents (Fig. 4) as they did when the cultures were kept at a temperature of 22–24° C. General exudation from the muciferous organelles was never seen when graphite was added, although a few hours after the addition practically every individual had ingested a certain amount—from minute particles to masses up to 4·5 μ (Fig. 12, Pl. I). Small discharges from the organelles were sometimes observed accompanying ejection of the graphite.

The actual ingestion of material occurs always at the non-flagellar pole (Fig. 12, Pl. I; Figs. 4, 5). The ingested material, if sufficiently small, is then moved close to one of the 'pyrenoids'. The whole process was followed in detail in a culture of *Chrysochromulina*, which had been cleaned by utilizing the phototactic properties of the species (Droop, 1954), until the bacterial contaminants were reduced to one species, in this case a species distinctly bottle-shaped and about 1 μ in length. The *Chrysochromulina* was then observed to take in bacteria by surrounding them with a clear or slightly granular substance which flowed out from the body enclosing one or more bacteria. Almost immediately afterwards the bacteria could be detected in a vacuole (Fig. 3) close to one of the 'pyrenoids' which were sometimes masked by the vacuole (Fig. 5). The bacteria then began to show dancing movements inside the vacuole and in a matter of 2–3 minutes were broken up into minute granules (Fig. 5) which continued to show Brownian movement for several more minutes. Similar vacuoles, full of minute particles in Brownian movement, were seen by Parke (1949) in *Chromulina pleiades*, but for *Prymnesium parvum* and *P. minutum*, Carter (1937) records the presence of a large number of minute granules in active Brownian movement in 'an ill-defined region', not in clearly delimited vacuoles. In this culture, with only one bacterial contaminant, the phagotrophic nature of the large amoeboid non-motile phase of the *Chrysochromulina* was also demonstrated; a number of vacuoles containing bacteria could be seen quite clearly lying close to the pyrenoid-like bodies (Fig. 7).

When the motile phase ingests cells with definite walls the cell contents are absorbed but the walls are not; a colourless tube containing the wall flows out, usually from the side of the body, and discards the wall from its tip (Fig. 4); the tube is then withdrawn into the body. Empty walls of *Chlorella*, *Stichococcus*, '*Stichochrysis*', *Porphyridium* and *Nitzschia gotlandica* have been seen thrown out of the body in this manner.

Reproduction follows the same pattern as that described for *Chrysochromulina kappa*, but in the motile phase no double-fission stages have so far been observed. The second haptonema and the two new flagella can be formed before the cell broadens for the actual fission, which can produce daughter-cells of equal or very unequal size (Fig. 6). In the species previously described, the daughter-cells remain attached by a small connexion at the non-flagellar pole when the fission is nearly completed, but in *C. ericina* the connexion was frequently seen to be between the sides of the daughter-cells (Fig. 6) towards the flagellar pole.

At the peak of growth a culture produces 1½–2 million cells per ml. Non-motile stages, similar to those described in detail for *C. kappa*, have been observed, forming a dark olive-green to brownish skin on one side of the bottom of the flask after the peak of growth has been passed. The large amoeboid cells, up to $14 \times 9{\cdot}5\,\mu$, with four very finely lobed chromatophores, frequently show large numbers of ingested bacteria (Fig. 7) while the tetrads of walled daughter-cells (Fig. 8), each with 2 stellate or finely lobed chromatophores, were distinguishable by deeper pigmentation. The free, walled daughter-cells (Fig. 9), in which the pyrenoid-like bodies could sometimes be seen, were usually ovoid, measuring from $4 \times 2{\cdot}5\,\mu$ to $7 \times 4\,\mu$. They differed only from those previously described in having a slightly thicker wall which appeared faintly brownish and was delicately rugose on the outside, somewhat as described by Carter (1937) for *Prymnesium parvum*. A thick culture of the motile phase can be obtained from the non-motile phase in 6–9 days after addition of fresh culture medium, the dark skin disappearing from the bottom of the flask.

Chrysochromulina ephippium n.sp., Parke & Manton

(Gr. ἐφίππιον—a saddle)

Diagnosis

Motile cells showing considerable metaboly, approximately saddle-shaped when moving slowly or stationary, bell-shaped to spheroidal when swimming rapidly, 6–10 (exceptionally 4·5–12) μ in size (length of back of saddle). Two flagella and one haptonema arising close together from the ventral concave surface near to one margin in a centre line; flagella equal, smooth, gradually attenuated to a hair point (E. M. observation), usually heterodynamic, occasionally appearing homodynamic, 3 to 4 times cell size in length; the haptonema, thinner than the flagella, 12 to 14 (exceptionally 16) times body size in length when fully extended, a club-shaped tip but no obvious translucent sheath visible with the electron microscope. The periplast, pectic in nature, showing a covering of very thin transparent circular to oval sculptured, dimorphic scales, visible only under the electron miscroscope. Scales without spines 0·5–0·7 μ, with a pattern of radiating ridges on one side and crossed striations within a wide raised rim on the other. Scales with spines 0·3–0·6 μ, with a pattern of radiating ridges on the side towards the body and a narrow raised rim surrounding concentric markings on the outer side, the slender tapering spine, approximately equal to scale diameter in length, attached by 4 decurrent ridges extending to scale margin. Distribution of two types of scales on body unknown.

Cells uninucleate, no stigma. Chromatophores appearing striated, 1 or 2, pale golden brown; in cells of motile phase parietal, saucer-shaped to oblong, with a single inconspicuous globular body (pyrenoid?) placed eccentrically on inner face of each; in cells of non-motile phase coarsely lobed. Oil and leucosin produced. Ejectile muciferous bodies small, localized in groups in peripheral cytoplasm, but their position changing with the metaboly of the body. Nutrition phototrophic and/or phagotrophic. Not toxic to fish.

In motile phase asexual reproduction by fission into two daughter-cells, usually of equal size. In non-motile phase by successive fission of amoeboid cells to produce 4 ovate daughter-cells with very thin walls; motile phase almost certainly liberated from walled daughter-cells through a pore.

Habitat: the sea at position (Plymouth Laboratory Station L4) Lat. N. 50° 15′, Long. W. 4° 13′ (13 Sept. 1950, type culture) from a townet sample. Type culture (Plymouth no. 31) deposited with the Type Culture Collection, Cambridge; preserved material and photographs lodged with the Marine Biological Association, Plymouth, England.

Cellula motili formam satis mutanti, fere ephippioidea cum lente natat aut restat immotilis, cupuliformi aut sphaeroidali cum natat rapiditer, longitudine 6–10 μ (rare 4·5–12 μ) per dorsum ephippii. Flagellis duobus et unico haptonemate conjunctim exorientibus ex aspectu concavo ventrali, prope lineum medium; flagellis aequis, teretibus, paulatim attenuatis sicut ad capilli extremitatem, ut videtur per microscopiam electronicam, generaliter heterodynamicis, nonnunquam homodynamicis, longioribus 3 vel 4 quam cellula. Haptonemate teneriore quam flagellis, 12–14 (rare 16) longiore quam cellula, cum maxime extensus, apice clavato sed nulla tunica externa semidiaphana ut videtur per microscopiam electronicam. Periplasto, pectico natura, induto delicatissimis squamis diaphanis, circularibus aut ovalibus, sculptis, invisibilibus nisi per microscopiam electronicam; squamis manifestis sub duabus formis: altera forma, sine spinulis, 0·5–0·7 μ longis, sculptis radiantibus rugis ad aspectum inferiorem, et striis decussatis circumdatis ab lata margine elevata ad aspectum superiorem; altera forma, praeditis spinulis longis 0·3–0·6 μ, sculptis rugis radiantibus ad aspectum inferiorem, et angusta margine elevata circumdanti rugas concentricas ad aspectum superiorem. Spinulis teneribus, longitudine ferme aequis latitudine squamae, affixis per quattuor costas decurrentes, extendentes ad marginem squamae. Ignotum est quomodo duae formae squamarum distributae sint in superficie cellulae.

Nucleo unico, nullo stigmate, chromatophoris striatis ut videntur, 1 aut 2, pallide aureo-brunneis; in cellula in statu motili, crateriformibus aut oblongis, praeditis unicis corporibus globularibus inconspicuosis (?pyrenoidalibus) locatis ex centro in aspectu concavo; in cellula in statu non-motili rude lobatis. Cellula oleum leucosinumque parienti; corporibus parvis ejectilibus et muciferosis, aggregatis in cytoplasmate superficiali, situ tamen mutanti secundum mutationem formae cellulae. Nutritione phototrophica necnon phagotrophica. Non toxica piscibus.

Generanti asexualiter in statu motili per fissionem in duobus cellulis filiolis, generaliter aequis magnitudine; in statu non-motili per fissiones subsequentes cellularum amoeboidalium ad 4 cellulas filiolas producendas, cum tenerrimis parietibus. Ferme certum est quod cellulae in statu motili ex cellulis filiolis per foramen liberantur.

Habitat mare prope Plymouth ad locationem Lat. N. 50° 15′, Long. W. 4° 13′ (13 Sept. 1950—cultura typica).

Description

The position of the haptonema differs from all those previously described in lying across the body during slow swimming (though not during rapid swimming) instead of projecting out from it. In this condition, or when anchored, the cells are roughly saddle-shaped with smooth curved sides. When seen from above or below the cells appear squarish or oblong with the

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Legends to Text-figs. 20–29

Chrysochromulina ephippium n.sp.

(Figs. 20–22 × 1250; Figs. 23–29 × 5000)

Fig. 20. Cell in shape adopted during rapid swimming; flagella and haptonema behind body in position characteristic for the species in this state.

Fig. 21. Saddle-shaped cell gliding slowly with haptonema fully extended in front of the body.

Fig. 22. Similar to Fig. 20 but sides of saddle overlapping differently and flagella in position for slower movement.

Fig. 23. Ventral view (concave surface) of saddle-shaped cell, haptonema loosely coiled; one flagellum still, the other undulating. *c*, chromatophore; *f*, flagellum; *g*, graphite; *h*, haptonema; *l*, leucosin vesicle; *m*, muciferous body; *n*, nucleus; *p*, pyrenoid-like body; *s*, scale; *v*, vacuole containing ingested particles in Brownian movement.

Fig. 24. Lateral view of saddle-shaped cell, haptonema fully extended; bacterium in vacuole.

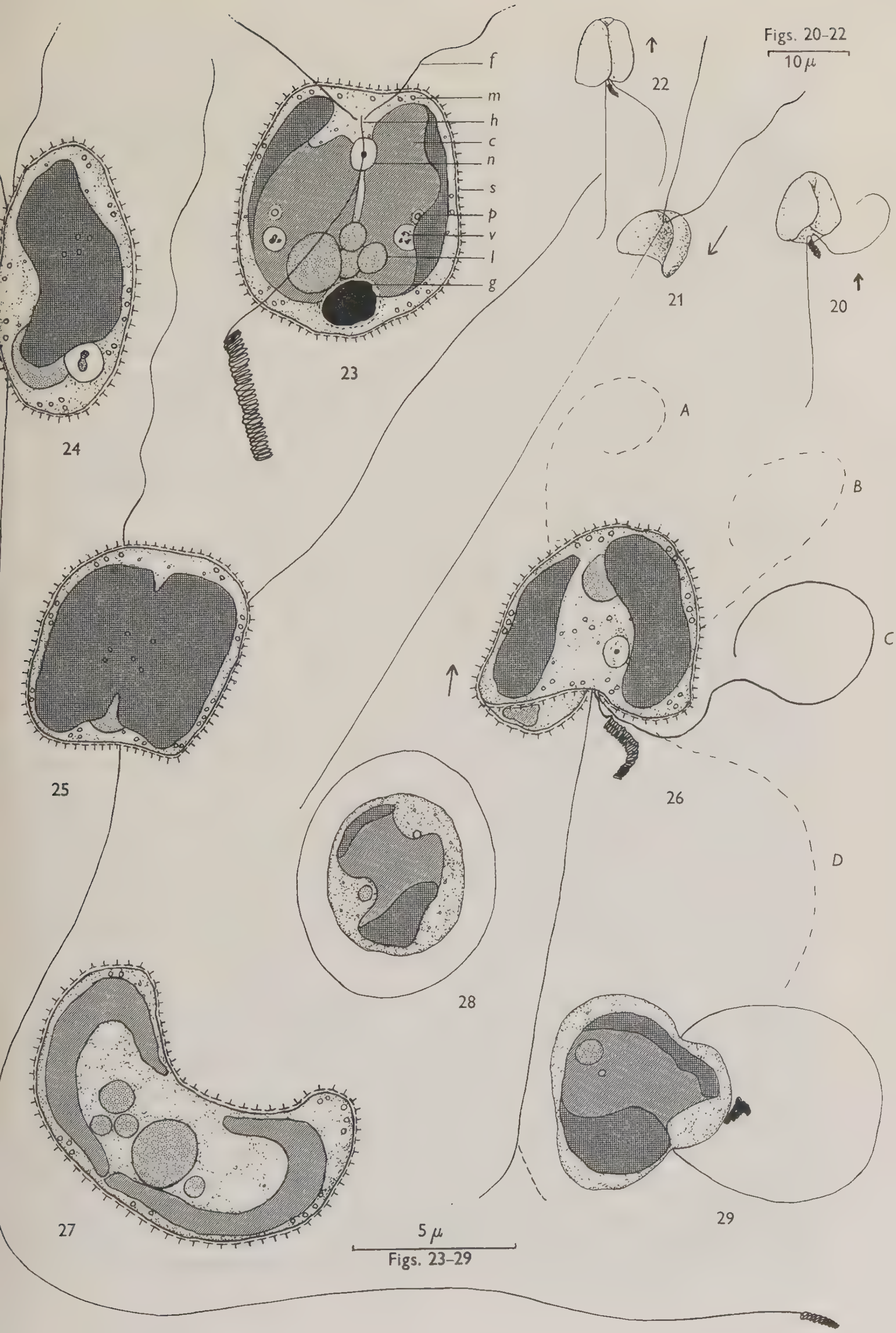
Fig. 25. Dorsal view (convex surface) of anchored saddle-shaped cell, chromatophore dividing, anchored haptonema bent and partly extended; flagella undulating at different speeds.

Fig. 26. Individual with sides of saddle overlapping; flagella and haptonema behind body in position characteristic for the species during very rapid swimming; positions *A* and *B*, less rapid swimming than in position *C*; position *D*, slower movement than in positions *A* or *B*.

Fig. 27. Optical section of large saddle-shaped individual with two chromatophores.

Fig. 28. Walled daughter-cell with contents shrunk away from the wall and with chromatophore similar to those of the motile phase.

Fig. 29. Contents of walled daughter-cell partly released through pore, flagella not detected.


Figs. 20–22
10 μ
22
21
20
f
m
h
c
n
s
p
v
l
g
23
24
A
B
C
25
26
D
28
27
29
5 μ
Figs. 23–29

Text-figs. 20–29

flagella and haptonema arising in the median line near one end of the concave ventral surface (Fig. 23). The lateral views (Fig. 24) are somewhat oval, the narrowest dimension being about half that of the dorsal or ventral views (Figs. 23, 25). The optical section through the saddle is bean-shaped (Fig. 27).

When the cell starts to swim rapidly the shape changes to a half-ovoid, or is spheroidal, or somewhat bell-shaped, with the flagella and haptonema directed backwards (Figs. 20, 22, 26). The change in shape is brought about by the rolling in and sometimes overlapping of the curved sides of the saddle. The different shapes during rapid swimming depend on how tightly the curved sides of the saddle are lying over each other. The flagellar end, now at the back of the saddle, sometimes protrudes behind the body as a small lobe (Fig. 20), the insertion of flagella and haptonema being clearly seen.

Using the length of the dorsal surface as an indication of size, in an actively growing culture 80% of the cells are from 6 to 9 μ, while 5% are between 4·5 and 6 μ. The remaining 15% are incipient fission stages and are from 9 to 12 μ, the largest being ovoid to nearly spheroidal in shape.

The flagella and haptonema (Figs. 30–32, 34, Pl. V; Figs. 35, 36, Pl. VI) are delicate and not very easily seen under the light field; they are also thrown off fairly quickly under both light and dark fields. Measurement of the flagella of a large number of individuals shows that the two flagella are of equal length although their movement is generally heterodynamic; they are 3–4 times the body size in length (Figs. 30–32, Pl. V; Figs. 20, 25, 26). The haptonema (Pl. V) is usually about four times the flagella length when fully extended (Fig. 21), but when very tightly coiled the coil measures 1·5–2·0 μ in length; the regular coiling, when not too tight, can be seen quite clearly under a 2 mm. objective (Fig. 23).

The general shape of the body scales, their surface sculpturing and relative sizes are shown on Pls. VI and VII. The spines are directed outwards (Fig. 35, Pl. VI) and the two differently marked surfaces of the subtending scale can thus be identified. On the inwardly directed surface (i.e. that away from the spine) there is the usual system of radiating ridges extending to the margin that has been encountered in other forms (e.g. *C. kappa*, *C. minor*, *C. ericina*). On the outer surface from which the spine arises there is a raised rim and roughly concentric surface markings upon which four cruciform ridges, extending from rim to centre, support the base of the spine. These two surfaces are separately distinguished in Fig. 39, Pl. VII, and in various parts of Fig. 38. Similar details for the two surfaces of spineless scales are contained in Fig. 40. By analogy with the spined scales it is probable that the rimless ridged surface (right-hand scale of Fig. 40) is that towards the body and that the face with the wide rim and criss-cross marking (left-hand scale of Fig. 40) is outwards. These scales are larger and fewer than the spined scales in this species (cf. Fig. 37, Pl. VI) but their relative distribution is unknown. It is probable that the information regarding the identity of the two surfaces will be found applicable to other species in which direct evidence has not so far been obtained, notably for the flat scales of *C. ericina*.

The chromatophores, one in smaller individuals, two in larger, are clearly striated and their position changes very considerably with the metaboly of the body. When the cell is saddle-shaped the chromatophores lie close to the dorsal surface (Figs. 23, 25) and curve round on to the ventral surface (Figs. 23, 27). They sometimes appear ribbon-shaped, the two edges nearly meeting ventrally if there is one chromatophore, or both dorsally and ventrally if there are two. When the cell changes shape for rapid swimming, the chromatophores tend to elongate, also becoming narrower (Fig. 26).

Completely colourless cells have not yet been detected in this species, but some peculiar small orange-brown chromatophores borne singly in a few otherwise unpigmented small cells are suspected to have been ingested fragments of degenerating cells. It is therefore probable that specimens lacking chromatophores are occasionally formed.

In the motile phase the pyrenoid-like bodies are very inconspicuous, measuring only about $0{\cdot}5\,\mu$ in diameter. In many individuals they could not be seen at all, but when observed each one appeared to lie on the inner face of a chromatophore towards one margin and slightly towards the non-flagellar end of the cell (Fig. 23); they sometimes appeared to be surrounded by a small mass of non-refringent material. The medium-sized nucleus is occasionally visible in the living cells lying in the body towards the ventral surface near the point of insertion of flagella and haptonema. In this species fairly small vesicles of leucosin are produced, generally one to three in each cell, and they lie in the body towards the dorsal surface away from the flagellar end (Figs. 23–25). Small oil globules could also be detected distributed throughout the cytoplasm. The refringent ejectile muciferous bodies are not very conspicuous and are localized in groups of 5 to 7, scattered over the body in the peripheral cytoplasm (Figs. 23–26). Their contents are generally exuded quickly as short threads, but sometimes slowly as small globules.

Movement is generally extremely rapid, the individuals swimming in straight lines for long periods. In spite of the absence of a stigma there is a marked phototactic reaction.

Figs. 20, 22 and 26 illustrate the body shape and the position of the flagella and haptonema during the most rapid swimming. The body rotates very quickly as the cell moves forward in the water, showing only slight gyration. One flagellum trails behind the body showing little movement except possibly near the tip, which appears to beat from side to side, though this appearance is probably due to the rotation of the body. The other flagellum adopts the various attitudes labelled *A* to *D* in Fig. 26, the position *C* being that of the most rapid motion, *A* and *B* being less rapid and *D* still slower. During very rapid swimming the haptonema is coiled. The various degrees of uncoiling exhibited by Figs. 30–32, Pl. V, are probably fixation effects but the attitudes of the flagella shown in these figures are highly characteristic of the normal slow swimming in the directions indicated by the arrows.

By putting the flagella straight out stiffly from the body, a cell can stop abruptly from rapid swimming. It then usually reassumes the saddle shape and becomes anchored by the end of the haptonema. Under dark field the uncoiling of the haptonema can be followed quite easily. It unrolls, sometimes quite slowly, the unrolling starting from the body until it lies out stiffly like a rod: it then attaches. Alternatively the haptonema can anchor at any stage of the uncoiling or when not uncoiled at all. If the haptonema does not uncoil when the cell anchors, the cell rotates very rapidly with the flagella either lying out from the body or curved inwards at their distal ends and under dark field recalling a catherine wheel firework. If the haptonema uncoils partly or completely the body is seen usually with the dorsal (convex) surface uppermost, the flagella projecting in the opposite direction to the haptonema (Fig. 25). When the cell is attached with the haptonema extended both

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Explanation of Plates V–VII

Chrysochromulina ephippium n.sp.

V

Fig. 30. A cell killed with iodine in KI and dried on a glass slide; photograph of the dry specimen taken without a coverslip. Magnification × 1000.

Fig. 31. Another cell, as Fig. 30.

Fig. 32. Another cell as Fig. 30 after transfer from glass to a quartz slide, examined in a liquid mount (water, with a trace of iodine) and photographed on the ultraviolet microscope with a glycerine-immersion monochromet (wave length 2750 A) Exposure no. UV 256·3*b*. Magnification × 3000.

Fig. 33. A cell killed with osmic vapour after graphite feeding, photographed in the cultur fluid with an oil-immersion lens. Magnification × 2000.

Fig. 34. A cell killed on a formvar film with osmic vapour, shadowed, and examined with the electron microscope; the appendages more disarranged than in Figs. 30–32 but visible. Electron micrograph M 115·20, magnification × 3000.

VI

Fig. 35. Central portion of the cell of Fig. 34, Pl. V, more highly magnified to show scales in position on the body. Electron micrograph M. 115·21, magnification × 10,000.

Fig. 36. Tip of a flagellum. Electron micrograph M. 273·11, magnification × 10,000.

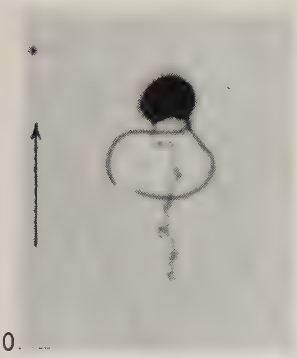
Fig. 37. Scales near the body of another cell. Electron micrograph M. 251·15, magnification × 20,000.

VII

Fig. 38. Scales near the body of another cell showing details of both surfaces of plate scales and spined scales; for further description see text p. 402. Electron micrograph M. 251·4, magnification × 30,000.

Fig. 39. Details of two spined scales from the field of Fig. 37, Pl. VI, to show the two different faces, left-hand scale showing inner face, right-hand scale showing outer face. Electron micrograph M. 251·15, reversed print, magnification × 30,000.

Fig. 40. Details of two plate scales from another cell showing the two faces, left-hand scale showing outer face, right-hand scale showing inner face. Electron micrograph M. 251·8, reversed print, magnification × 30,000.



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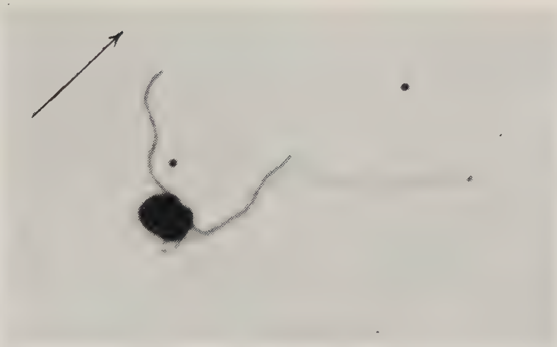

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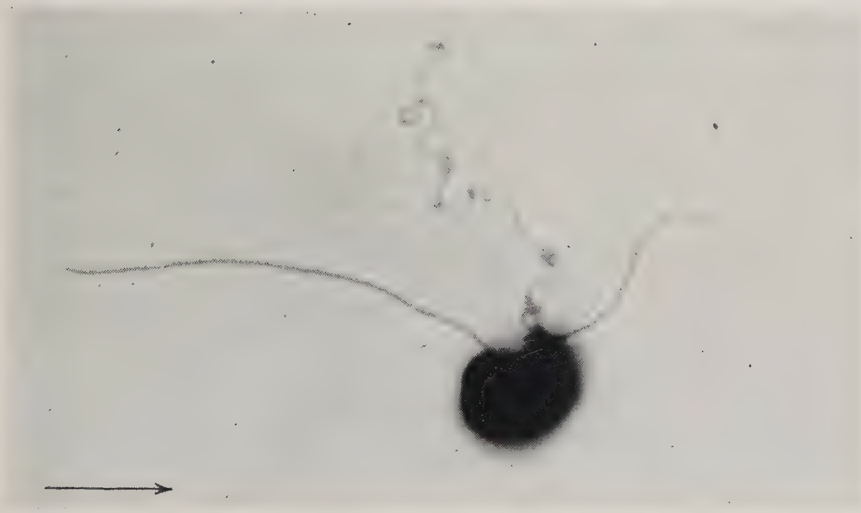

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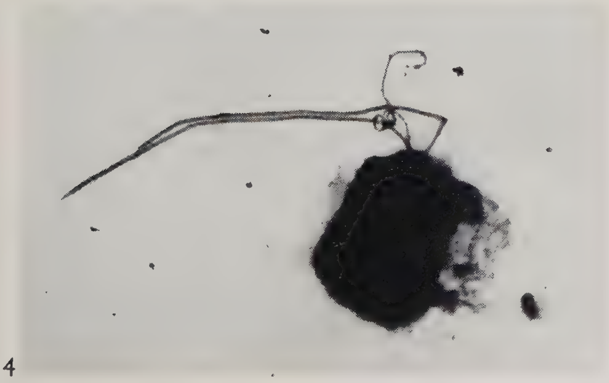

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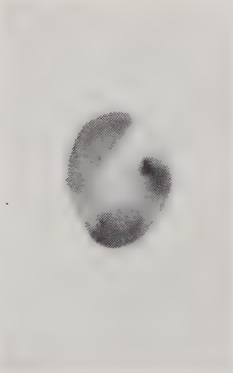

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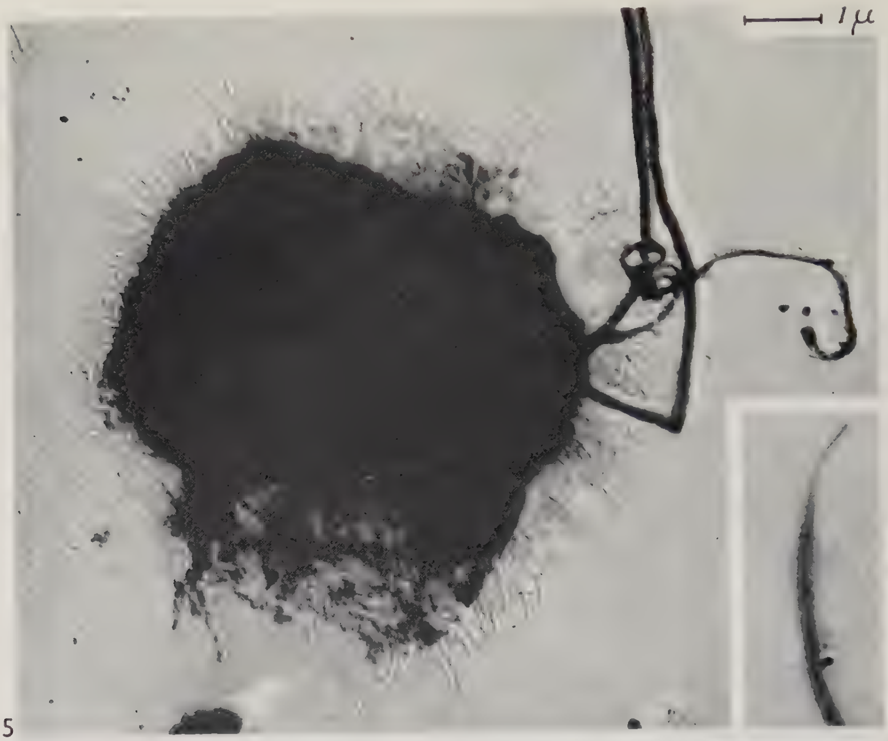

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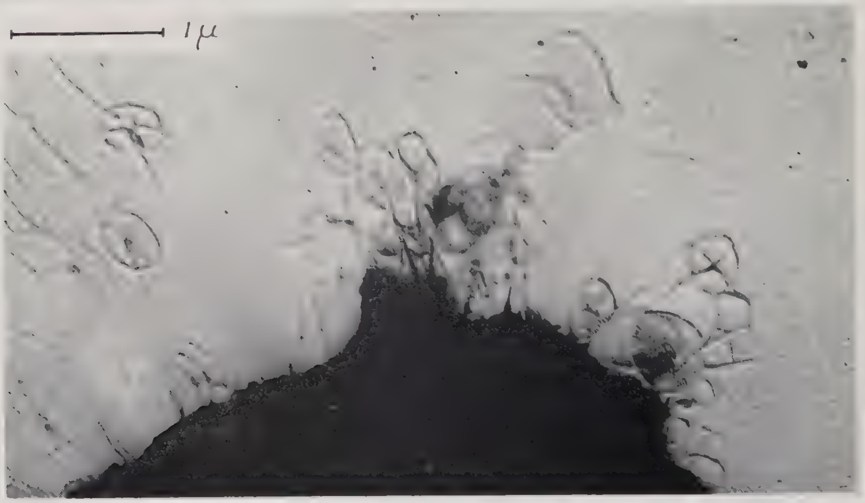

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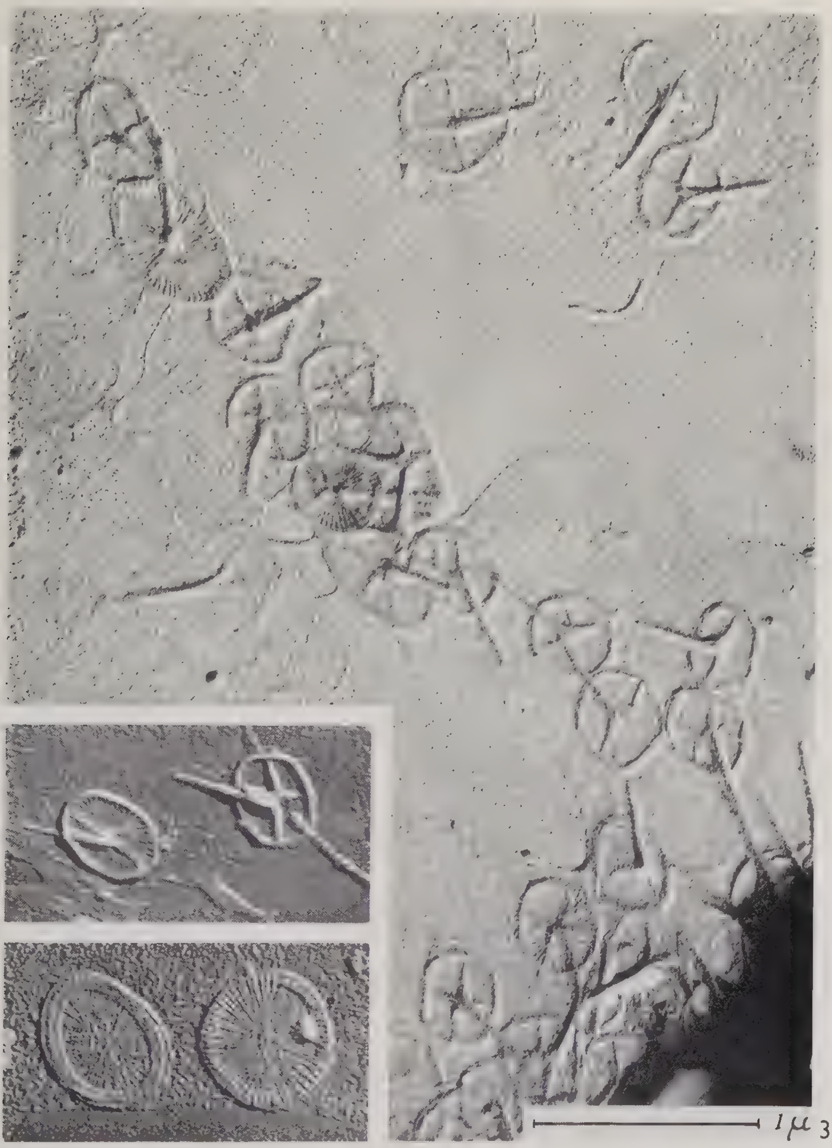

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flagella can undulate at the same rate, or appear to do so, or one can undulate more slowly than the other with undulations of larger amplitude (Fig. 25). In the extreme case, which is quite common, one flagellum can undulate and the other remain still or move stiffly in a short back and forward dithering stroke. When attached with the haptonema extended the body can sway about on the attached haptonema and the haptonema itself can bend over (Fig. 25) so that sometimes the body is near the point of attachment of the haptonema.

Saddle-shaped cells are frequently seen gliding through the water and rotating very slowly with the haptonema fully extended forwards and lying across the body in the direction of motion (Figs. 21, 24). The flagella thus project backwards and both can either undulate slowly, usually at a slightly different rate, or one can remain stiff while the other undulates (Fig. 21). If another cell is encountered, or for no apparent reason, the haptonema may be withdrawn with a sudden jerk and coiled up so quickly that the act cannot be followed. The body then resumes the shape and characteristics of rapid swimming.

Phagotrophy is of common occurrence, the cells ingesting graphite, bacteria and other organisms up to a size of 2·5 μ. Ingested material lies at the non-flagellar end towards the dorsal surface (Figs. 23, 24). In a few instances small vacuoles containing either bacteria or graphite have been seen lying close to the pyrenoid-like bodies (Fig. 23). As in *C. ericina*, Brownian movement can be seen inside the vacuoles which, after a short time, suddenly disappear.

Before fission the motile cells become more ovoid to spheroidal in shape, the incipient fission stages being from 9–12 μ in diameter. The second pair of flagella, frequently seen as very short ones, and the second haptonema develop before the actual fission which passes from dorsal to ventral surface, giving usually daughter-cells of equal size but occasionally ones of unequal size.

In culture, this species produces from 1 to 2 million cells per ml. at the peak of growth, after which, as in the other species, non-motile stages are produced. The large naked phagotrophic amoeboid cells, up to 16 × 9 μ, have lobed chromatophores but they are rather coarsely lobed in this species. The four daughter-cells with stellate chromatophores, product of the fission of the large walled cells up to 14 × 10 μ, are generally ovoidal with a very thin smooth wall, and range in size from 5 × 3·5 to 8 × 6 μ. In a four-month-old culture many of these small, walled, cells were present and in some of them the contents had shrunk quite considerably leaving a clear area inside the wall. The cells with the shrunk contents (Fig. 28) were more deeply pigmented than the others and therefore conspicuous. On examination it was found that the chromatophores although striated were no longer lobed but had resumed the appearance of those of the motile phase (Figs. 28, 29); the pyrenoid-like body and small leucosin vesicles could also be seen in some of these cells. Only the part-release of the contents of a number of these daughter-cells has been seen so far; in one, the cell was seen to come partly out of the wall through the pore and what was almost certainly the haptonema could be seen on that part of the body still inside the wall, but no flagella could be detected. Numerous empty walls with circular pores were found on the bottom of the flask containing the four-month-old culture.

In shape this species is very similar to the type species of the genus, *C. parva* Lackey, but it is larger, has a relatively longer haptonema which is thinner instead of thicker than the flagella. It also lacks a contractile vacuole and, when saddle-shaped, has the flagella and haptonema projecting in the opposite direction to that shown by Lackey for *C. parva*.

Chrysochromulina alifera n.sp., Parke & Manton

(Lat. *Ala*—a wing + *fero*—I bear)

Diagnosis

Motile cells showing extreme metaboly, approximately saddle-shaped with large lateral curved wings when moving slowly or stationary; bell-shaped, oblong, ovoid or spheroidal when swimming rapidly; 6–10 (exceptionally 4–12) μ in length of back of saddle. Two flagella and one haptonema arising close together from ventral concave surface near to one margin in a centre line; flagella smooth, of equal length or subequal, gradually attenuated to a hair point (E. M. observation), usually heterodynamic, occasionally appearing homodynamic, 2–2½ times cell size in length; the haptonema thinner than the flagella, 10 to 12 (exceptionally 14) times body size in length when fully extended, with a swollen tip but no obvious translucent sheath visible under the electron microscope. The periplast, pectic in nature, showing a covering of very thin transparent circular to oval sculptured, dimorphic scales, visible only under the electron microscope; scales without spines 0·25 to 0·45 μ, sculpturing similar to those of *C. ephippium*; scales with spines 0·28 to 0·45 μ, the spine slightly less than scale diameter in length attached centrally by 2–4 short decurrent ridges not extending to the margin. Distribution of the two types of scales on body unknown.

Cells uninucleate, no stigma. Chromatophores striated, 2 or 4, occasionally one or none, intense golden brown; in cells of motile phase saucer-shaped to square or oblong, with single inconspicuous globular body (pyrenoid?) placed near the margin towards the non-flagellar end; in non-motile phase finely lobed. Oil and leucosin produced. Ejectile muciferous bodies small, localized in groups mainly towards the non-flagellar end of the cell. Nutrition phototrophic and/or phagotrophic. Not toxic to fish.

In motile phase asexual reproduction by fission into two daughter-cells of equal or unequal size; in non-motile phase by successive fission of amoeboid cells to produce 4 ovate daughter-cells with exceptionally thin walls; motile phase probably liberated from walled daughter-cells through a pore. Habitat: the sea at position (Plymouth Laboratory Station L4) Lat. N. 50° 15′, Long. W. 4° 13′ (4 May, 1950, type culture) at surface. Type Culture (Plymouth no. 34) deposited with the Type Culture Collection, Cambridge; preserved material and photographs lodged with the Marine Biological Association, Plymouth, England.

Cellula motili, maxime formam mutanti, fere ephippioidea, praedita magnis alis lateralibus curvatis cum lente motilis aut immotilis, cupuliformi, oblonga, ovoidali aut sphaeroidali cum natat rapiditer; longi 6–10 μ (rare 4–12 μ) per dorsum ephippii. Flagellis duobus et haptonemate unico conjunctim exorientibus e concavo aspectu ventrali prope marginem in medio lineo; flagellis teretibus, longitudine aequis aut subaequis inter se, paulatim attenuatis sicut ad capilli extremitatem ut videtur per microscopiam electronicam; generaliter heterodynamicis, nonnunquam homodynamicis, ut videtur, longioribus 2–2½ quam cellula; haptonemate teneriore quam flagellis, longiore 10–12 (rare 14) quam cellula, cum maxime extensus, apice tumescenti sed nulla tunica externa semi-diaphana apparente, ut videtur per microscopiam electronicam. Periplasto, pectica natura, induto delicatissimis diaphanis squamis circularibus aut ovalibus, sculptis, manifestis sub duabus formis, invisibilibus nisi per microscopiam electronicam; altera forma, squamis sine spinulis, 0·25–0·45 μ longis, sculptis simili modo squamis *C. ephippii*; altera forma, squamis praeditis spinulis, longis 0·28–0·45 μ quoque spinulo longo minusquam squamae latitudo, affixo ad centrum squamae per 2–4 breves costas decurrentes, non extendentes usque ad marginem squamae. Ignotum est quomodo duae formae squamarum distributae sint in superficie cellulae.

Nucleo unico, nullo stigmate. Chromatophoris striatis, 2 aut 4, rare unico aut absente, profunde aureo-brunneo; in cellulis in statu motili, crateriformibus, rectangularibus aut oblongis, unico corpore inconspicuoso globulari (?pyrenoidali) locato prope marginem cellulae, versus apicem quo desunt flagella; in cellulis in statu non-motili chromatophoris delicate lobatis. Corporibus ejectilibus muciferosis parvis, locatis versus apicem cellulae quo desunt flagella. Nutritione phototrophica necnon phagotrophica. Non toxica piscibus.

Generanti asexualiter in statu motili per fissionem in duas cellulas filiolas magnitudine aequas aut inaequales; generanti in statu non-motili per fissiones subsequentes cellularum amoeboidalium ad 4 ovatas cellulas filiolas producendas, parietibus extreme delicatissimis. Fere certum est quod cellulae in statu motili liberantur per foramen.

Habitat mare prope Plymouth ad locationem Lat. N. 50° 15′, Long. W. 4° 13′ (4 Mai 1950—cultura typica) ad summum maris.

Description

The details which distinguish this species from the preceding include body shape, the relatively shorter flagella and haptonema, some details of the swimming movements, the position of the pyrenoid, the greater average number of chromatophores and the smaller and slightly simpler scales.

The exceptional form-range is illustrated in Figs. 41–59. In general character this species is somewhat similar to *C. ephippium*, though the form range is greater, the body thinner (Fig. 65) and the wings larger and more curved (Figs. 46, 60, 61). It was impossible to get an exact measurement of the thickness of the body, but it is not more than 1·5–2·0 μ.

When a cell glides slowly through the water without rotating and with the haptonema extended, or when it is anchored by the extended haptonema, the body shape is characteristically as in Fig. 60 (see also Fig. 70, Pl. VIII). When the cell moves fairly slowly with the haptonema extended and the body rotating the sides curl in slightly and frequently overlap (Figs. 41, 42). Sometimes the sides fold in, one above the other, as in Fig. 49. When the cells are swimming rapidly (Figs. 55–57, 62, 63) all shapes from spheroidal, ovoidal, half an ovoid, oblong, bell-shaped to umbrella-shaped can be seen, depending on how much and at what angle the wings are overlapping.

The flagella (Pls. VIII, IX) are comparatively shorter and a little sturdier than in *C. ephippium*, but even so neither the flagella nor the haptonema are easily seen under the light field, particularly when the cells are moving rapidly; the flagella are also thrown off very quickly under both light and dark fields. Measurement of the flagella of a large number of cells showed that in the majority they were of equal length, as in the previous species, but in a few, one flagellum was slightly longer (1–3 μ) than the other; their movement is generally heterodynamic as in *C. ephippium*. The haptonema is usually

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Legends to Text-figs. 41–59

Chrysochromulina alifera n.sp. (× 1250)

Fig. 41. Saddle-shaped cell moving slowly with haptonema fully extended, one flagellum slowly undulating, the other stiff or gently vibrating; wings incurved, one slightly overlapping the other.

Fig. 42. As Fig. 41 but wings incurved and not overlapping, body rotating slowly.

Fig. 43. As Fig. 41 but wings straight, body not rotating and haptonema lying close to ventral surface of body.

Fig. 44. As Fig. 41 but wings lying close together but straight not incurved, haptonema lying away from body.

Fig. 45. Anchored cell with haptonema coiled and flagella lying out from body, two new flagella developing, wings of body curved in and one slightly overlapping the other; body rotating very rapidly giving the impression of a catherine wheel firework when looking down on it under dark field.

Fig. 46. Stationary cell with wings straight out and haptonema slowly uncoiling.

Figs. 47–48. Cells anchored by partly extended haptonemata.

Fig. 49. Cell swimming with flagella and haptonema in front of the body, body elongated and wings rolled in one above the other.

Figs. 50–51. Stationary cells with incurved wings.

Fig. 52. Early fission stage with overlapping wings and with four flagella and two haptonemata behind body, characteristic position for rapid swimming.

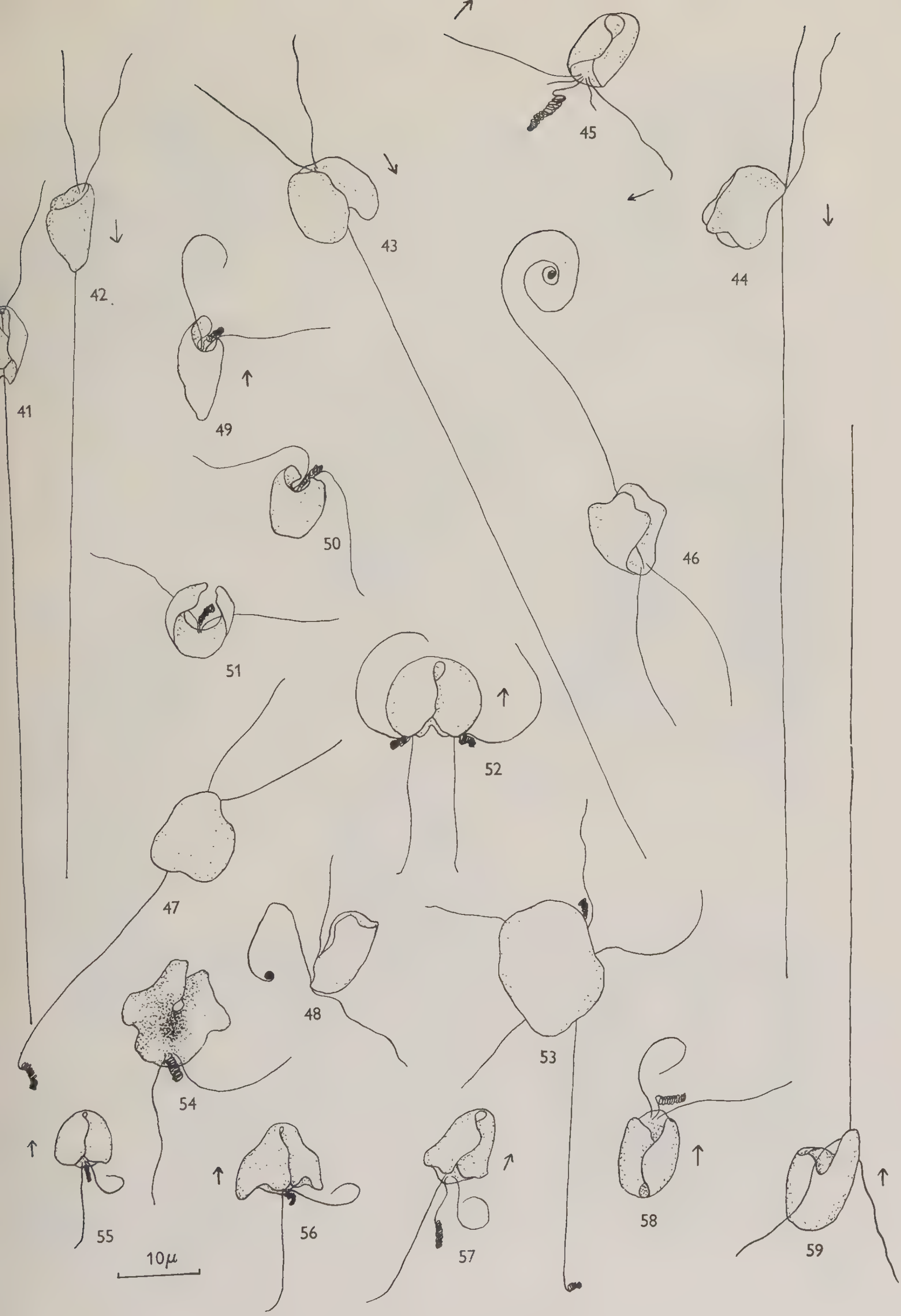
Fig. 53. Early fission stage with four flagella and two haptonemata, body anchored by one haptonema, the other coiled up close to body.

Fig. 54. Cell in process of overlapping wings to produce shape seen in Fig. 56 looking down on ventral surface which is folded inside in Fig. 56.

Figs. 55–57. Variously shaped cells with flagella and haptonemata behind body in positions characteristic for the species during rapid swimming.

Fig. 58. Cell swimming with flagella and coiled haptonema in front of the body.

Fig. 59. Same cell as Fig. 58 just stopped swimming, body shape slightly changed and haptonema extended. It then started gliding with the extended haptonema in front of the body.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Text-figs. 41–59

about five times the length of the flagella when fully extended (Figs. 41–44) and shows the same regular coiling (Figs. 61–64) as in *C. ephippium*, and is again thinner than the flagella (Fig. 70, Pl. VIII; Fig. 73, Pl. IX).

The scales (Pl. IX) are very much like those of *C. ephippium* but smaller, and their markings, especially those of the outer face, less distinct (Fig. 76, Pl. IX). The struts at the base of the spine are also less massive on the spined scales and they do not extend to the rim of the scale (Figs. 74, 76, Pl. IX).

The chromatophores, two in smaller individuals (Fig. 61), four in larger (Fig. 60), are striated, but not as clearly as in *C. ephippium*. Occasional cells, usually small, lacking chromatophores have been seen in stock cultures of this species and they were found to be not uncommon in cultures which had been treated with penicillin and streptomycin. When two chromatophores are present they lie mainly in the wings (Fig. 61), filling them completely and leaving most of the back as a clear area (Figs. 65, 66); when four are present (Fig. 60) two are then situated in this part and no clear area is visible but their shape and position changes with the metaboly of the body. Occasionally cells possessing extremely small chromatophores are seen, the chromatophores filling only about one quarter of the wings.

As in *C. ephippium* the pyrenoid-like bodies are small (Figs. 60, 63), 0·5–0·75 μ diameter, and cannot always be seen; their position is on the margin of the chromatophore towards the non-flagellar end of the cell (Fig. 66); they sometimes appear to lie on the margin in the centre line, but if the chromatophore is squarish or oblong they appear to be at one corner. The nucleus is of medium size and lies in the body close to the point of the insertion of the flagella and haptonema (Figs. 60, 63). As in *C. ephippium* a number of small, sometimes very small, vesicles of leucosin are produced, up to 5 in a cell, but no large vesicles have been observed. The leucosin lies in the central clear area

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Legends to Text-figs. 60–67

Chrysochromulina alifera n.sp. (× 5000)

Fig. 60. Saddle-shaped cell with straight wings and with the haptonema fully extended, the shape characteristic of gliding motion without rotation: four chromatophores and four pyrenoid-like bodies, ingested graphite at non-flagellar end of back of saddle.

Fig. 61. Saddle-shaped cell with two chromatophores and coiled haptonema.

Fig. 62. Cell in the shape adopted for rapid swimming, wings in the front, incurved and slightly overlapping; two chromatophores, one in each wing.

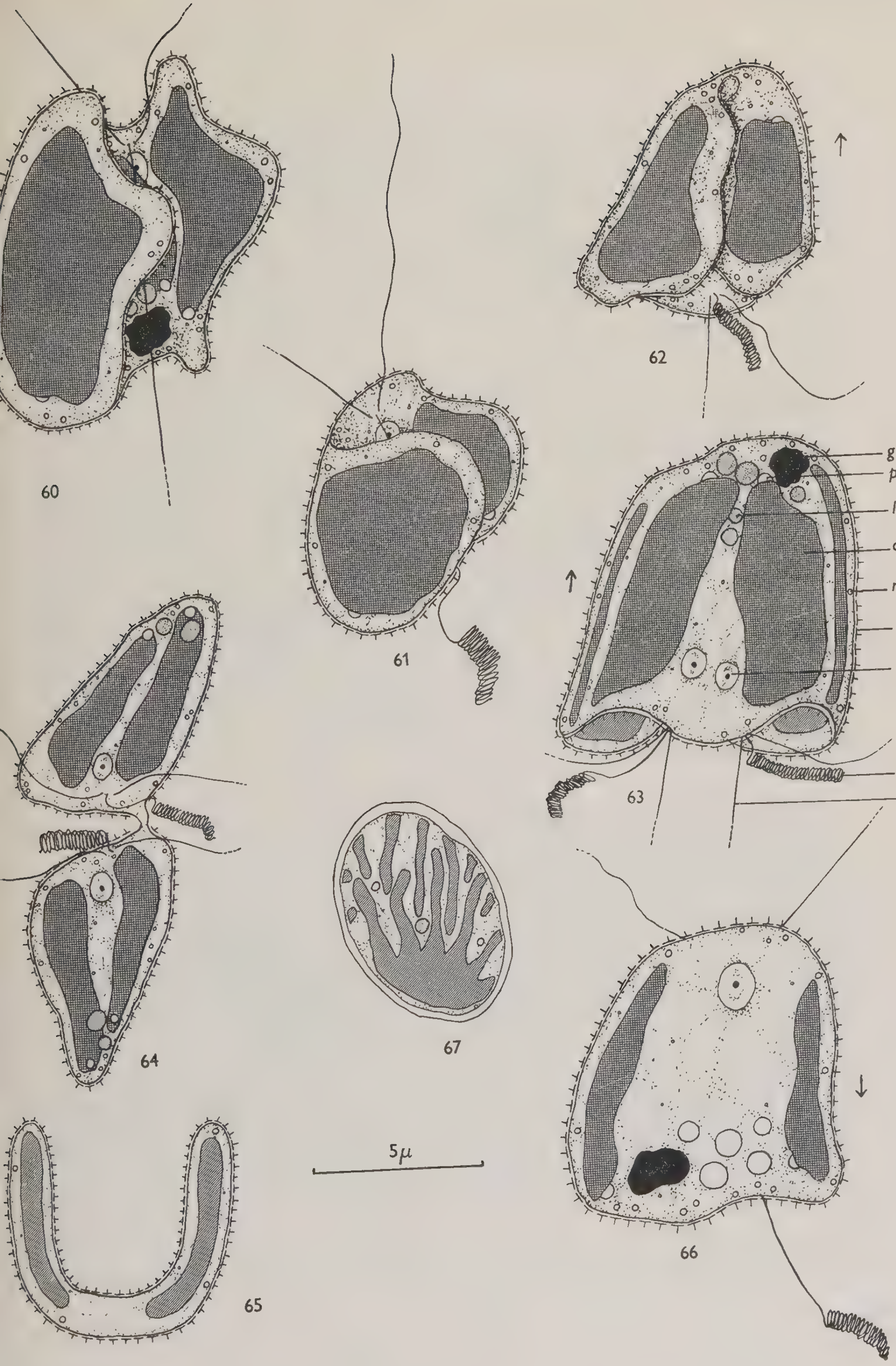
Fig. 63. Early fission stage with four chromatophores, four flagella, two haptonemata, and two nuclei; wings curved in and overlapping behind; ingested graphite at non-flagellar end of back of saddle. *c*, chromatophore; *f*, flagellum; *g*, graphite; *h*, haptonema; *l*, leucosin vesicle; *m*, muciferous body; *n*, nucleus; *p*, pyrenoid-like body; *s*, scale.

Fig. 64. Late fission stage.

Fig. 65. Optical section of saddle-shaped cell.

Fig. 66. Anchored cell viewed from convex dorsal surface, ingested graphite at non-flagellar end.

Fig. 67. Small thin-walled daughter-cell with two stellate chromatophores and two pyrenoid-like bodies.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Text-figs. 60–67

of the cell at the end opposite to that at which the flagella and haptonema are inserted (Figs. 63, 66); leucosin vesicles have not been detected in the lateral wings. Small oil globules are generally distributed throughout the cytoplasm. Inconspicuous muciferous bodies are present in small groups in the peripheral cytoplasm, and appear more numerous in that part of the body in which the leucosin is situated (Figs. 63, 66).

Movement is very rapid but the cells do not move quite so quickly as in *C. ephippium*, neither do they swim for such long periods in one direction; they do, however, show a marked phototactic reaction in spite of the absence of an obvious stigma. The behaviour of the flagella and their position during rapid (Figs. 55–57) and slow (Figs. 41–44, 49, 58, 59) movement is similar to that already described for *C. ephippium*; that is, the flagella nearly always behave heterodynamically, though sometimes appearing to be homodynamic.

The haptonema is sometimes seen partly extended behind the body (Fig. 57) during fast swimming, with the cells rotating very rapidly, sometimes showing considerable gyration and often changing their shape whilst in motion. Swimming with the flagella and haptonema in front of the body (Figs. 49, 58, 59) is more frequent than in *C. ephippium* but the movement is much slower than when the flagella are behind the body. The uncoiling of the haptonema (Fig. 46) and the method of anchorage of the cell by it (Figs. 47, 48) is as described for *C. ephippium*. The rapid rotation of the anchored body (Fig. 45), recalling under dark field the motion of a catherine wheel, occurs also in this species, but the rotation does not last for such long

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Explanation of Plates VIII–IX

Chrysochromulina alifera n.sp.

VIII

Fig. 68. A cell killed with osmic vapour and dried on glass, photographed without a coverslip. Magnification ×1000.

Fig. 69. A cell after graphite feeding, killed with osmic vapour and photographed in a liquid mount under oil immersion. Magnification ×2000.

Fig. 70. The cell of Fig. 68 stripped from glass and remounted for electron microscopy. Electron micrograph M. 162·1, magnification ×approx. 2300.

Fig. 71. Another cell killed directly on the formvar film. Electron micrograph M. 179·7, magnification ×3000.

IX

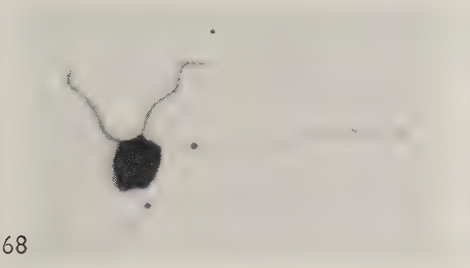
Fig. 72. Tip of a flagellum. Electron micrograph M. 273·11, magnification ×10,000.

Fig. 73. A body showing haptonema and scales. Electron micrograph M. 179·8, magnification ×10,000.

Fig. 74. Part of the field of Fig. 73 to show details of scales. Electron micrograph M. 179·12, magnification ×20,000.

Fig. 75. Group of scales more highly magnified showing spined and spineless scales, most viewed from the body side. Electron micrograph M. 277·12, reversed print, magnification ×30,000.

Fig. 76. Another part of the field of Fig. 75; an isolated spined scale showing the outer face on the right; both views visible in the group on the left. Electron micrograph M. 277·12, reversed print, magnification ×30,000.



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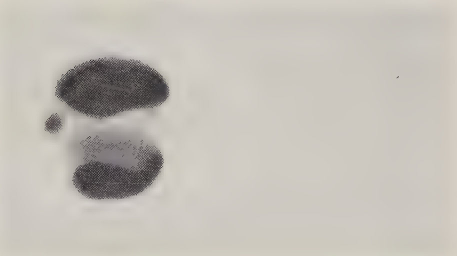

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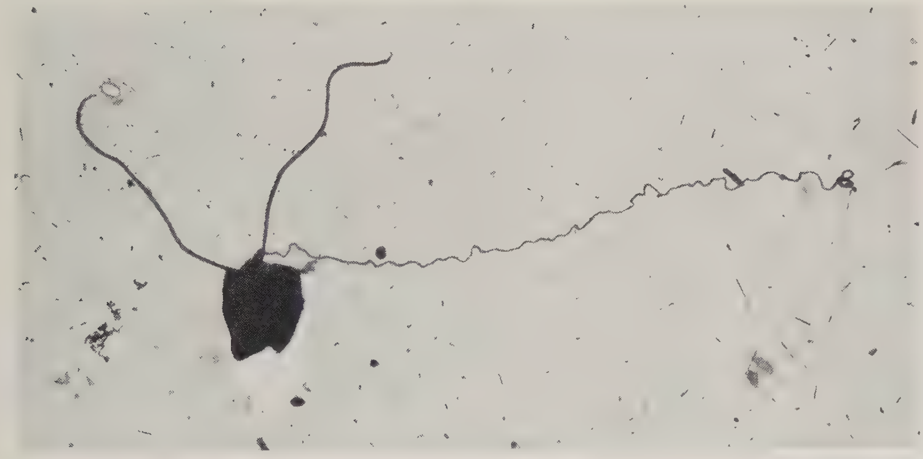

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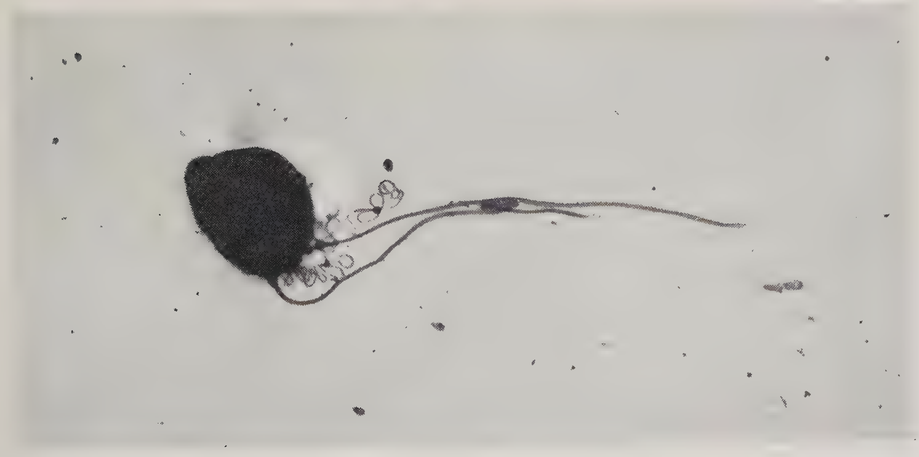

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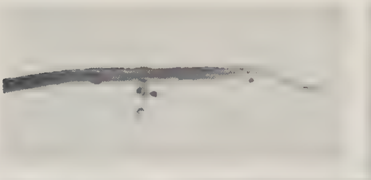

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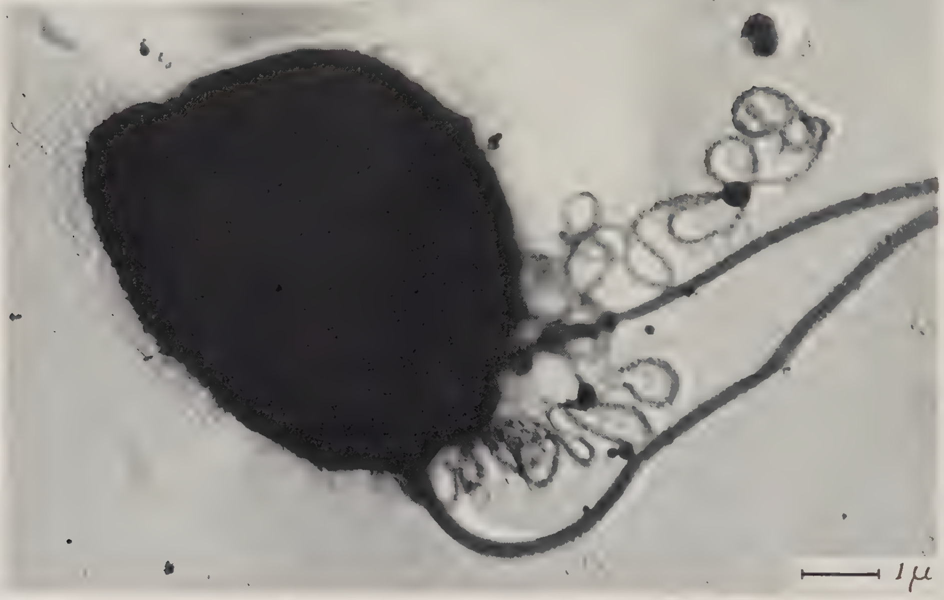

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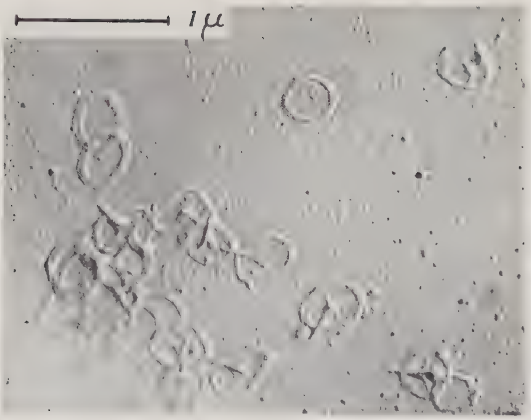

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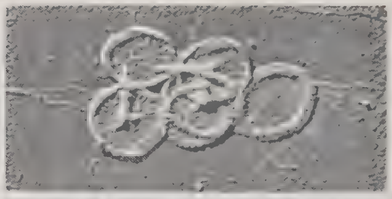

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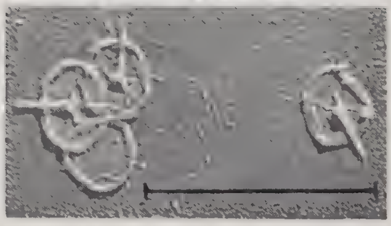

76

periods as in *C. ephippium.* Cells are commonly seen stationary, or gliding through the water with their haptonemata extended for longer periods than in *C. ephippium*, and, as in that species, the cells always give a sudden backward jerk when the haptonemata are coiled up rapidly.

Phagotrophy has been demonstrated (Fig. 69, Pl. VIII; Figs. 60, 63, 66). Cells containing ingested material are frequently but not commonly seen, the maximum size of ingested material being 2 × 1 μ. Ingestion of material takes place at the back end of the saddle (Fig. 60), that is the part of the body foremost (Fig. 63) when the cell is swimming rapidly with the flagella and haptonema behind. No vacuoles containing granules in Brownian movement have so far been seen.

Before fission the back of the saddle widens (Fig. 63), not lengthens, and cells showing two very short flagella and two long flagella (Fig. 45), are sometimes seen. The second haptonema, as well as the two new flagella, are formed before the actual fission starts (Figs. 52, 53, 63). In this species incipient fission stages are frequently seen anchored by one haptonema whilst the second remains coiled (Fig. 53). Fission down through the back of the saddle starting at the non-flagellar edge gives two daughter-cells (Fig. 64), which can be from equal to very unequal in size.

In culture from ½ to 1 million cells per ml. are produced at the peak of growth. Non-motile stages similar to those already described for the other species are then produced. The large amoeboid and walled cells, with chromatophores more finely lobed than in *C. ephippium*, measure from 14 × 10 to 16 × 12 μ. The four ovate daughter-cells produced by the large walled cell have exceptionally thin walls and fairly finely lobed or stellate chromatophores and measure from 4 × 3 to 7 × 5 μ in size (Fig. 67). The shrinkage of the contents of these cells away from the walls has not so far been seen in old cultures, nor has the liberation of the contents been observed.

Discussion

The only point which at this stage perhaps merits further discussion is our treatment of the facts for heterodynamic flagellar motion in *C. ephippium* and *C. alifera.* We are well aware that, on some systems of classification, this character would at once remove these species not only from the same genus but even from the same order as that containing the other species with which we have been concerned. That we have not, at this stage, chosen to do this is partly due to the striking similarity of all these species in other respects, which in this particular group are perhaps as important taxonomically, but in part also to our inability to find any structural differences between the two flagella of the kind which normally accompanies the truly heterokont condition, cf. *Synura* (Manton, 1955); when fixed therefore they cannot be distinguished from isokonts. There is the further difficulty caused by our present ignorance of the relevant facts for the type species *C. parva* Lackey. We do not yet know whether the apparent resemblance between this species and our *C. ephippium* extends to the motion of their flagella, and without this knowledge we should be in grave danger of misapplying the generic name were we to attempt to split the assemblage of species at present included under *Chrysochromulina* on a character as elusive as flagellar motion.

This negative attitude does not, however, preclude the possibility that subdivision may have to be carried out at a later stage. The six species which we have now described fall into three or perhaps four distinct assemblages, namely *C. ericina*; *C. ephippium* and *C. alifera*; *C. kappa* and *C. minor*; and *C. brevifilum*. We are not, however, yet prepared to say whether these ought to be thought of as subgenera or as genera, and since we have additional groups still undescribed, further discussion of the larger topic must necessarily be deferred.

SUMMARY

Diagnoses and descriptions are given of three new species of marine plankton flagellates in the class Chrysophyceae: *Chrysochromulina ericina*, *C. ephippium* and *C. alifera*. All possess two equal or subequal flagella and a long haptonema. In two of the species flagellar movement is heterodynamic and in one homodynamic. Phagotrophic feeding has been demonstrated in all. The descriptions include structural details of scale characters visible only with the electron microscope as well as observations on behaviour and life-history visible only in living material. The reasons for temporarily placing the three organisms in the genus *Chrysochromulina* Lackey are given.

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OBSERVATIONS ON THE SHOALING BEHAVIOUR OF COD (*GADUS CALLARIAS*) IN DEEP WATER RELATIVE TO DAYLIGHT

By G. H. Ellis

[Kelvin and Hughes Ltd]

(Plates I and II)

It is well known that vision is the main physical factor governing the formation and maintenance of fish shoals, and that, in general, shoals break up when the light intensity falls below a certain level. Breder (1929, 1942), Newman (1876), Parr (1927, 1931), and others, have shown this by laboratory experiment.

During a commercial fishing voyage to the Barents Sea it became possible, by means of a recording echo-sounder, to study the shoaling behaviour of cod in deep water relative to light intensity. The observations were made aboard the Hull trawler *Lancella* whilst fishing in a depth of 110 fathoms at Skolpen Bank in September 1955. For this a Kelvin and Hughes recording echo-sounder type MS. 24J was used, the depth range across the chart being 55 fathoms. The scale was phased so that the region between 80 and 135 fathoms deep was displayed.

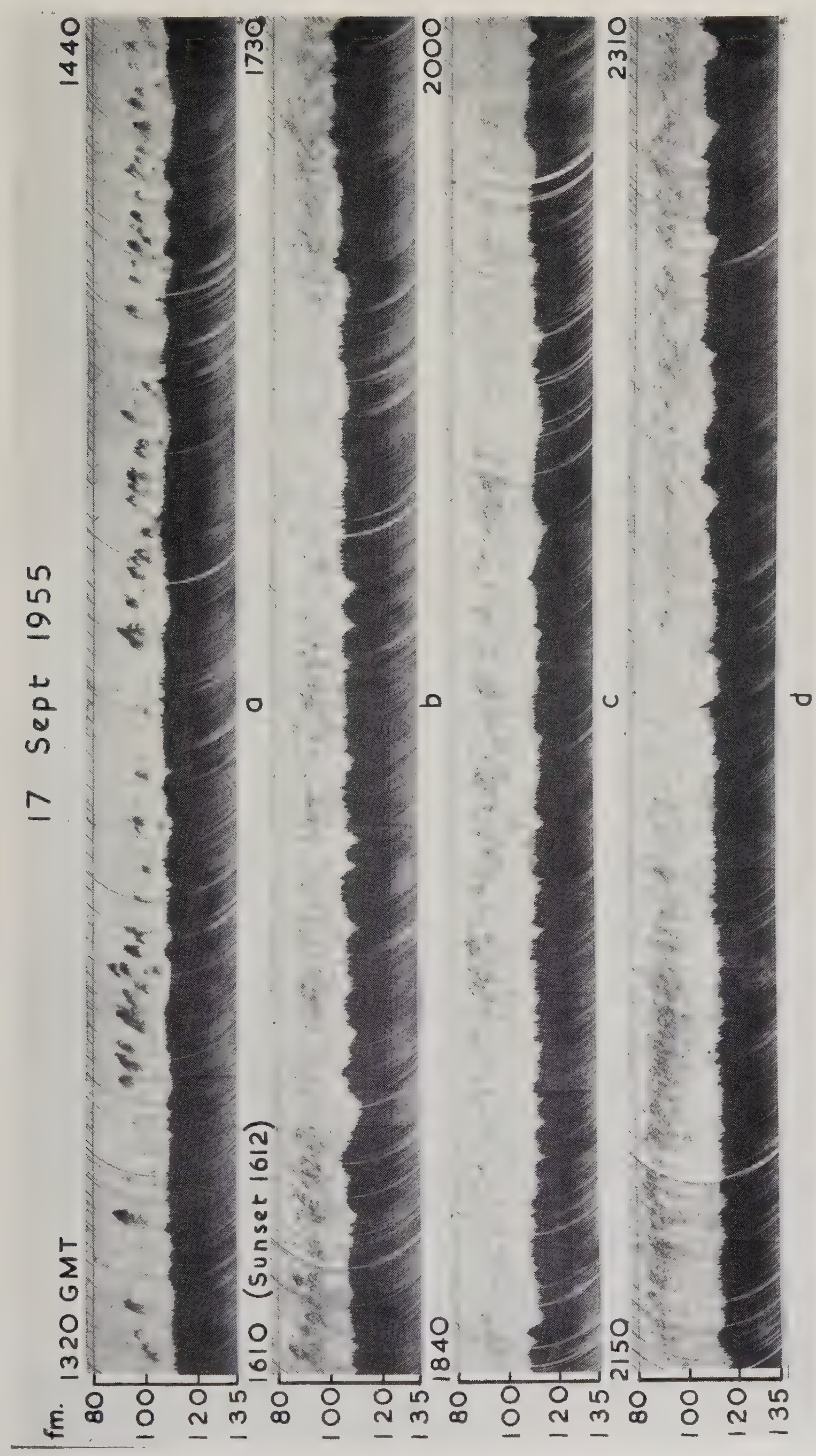
The trawler was, for the period of 19 h, towing over substantially the same ground on consecutive trawling tows, position and direction of tow being maintained by the use of radar equipment in conjunction with two anchored dan buoys. The duration of each tow was approximately 2 h. The fishing gear used was a standard deep sea trawl, and the towing speed of the vessel was 4 knots.

The charts (Pls. I, II) show the results obtained on seven consecutive trawling tows made between 13.10 G.M.T. on 17 September 1955, and 08.15 G.M.T. on 18 September 1955, for which particulars are given in Table I. It will be noticed that all recorded echoes have serrated edges, and that there is occasional 'missing' and 'lining' on the charts. These effects are caused by the vertical movement of the vessel due to heavy swell which persisted throughout the tows. All fish caught whilst these charts were recorded were cod, the length of which lay between 40 and 90 cm. From the catches it is assumed that the recorded fish traces were due to cod of sizes within this range.

The charts show clearly that the cod were mainly in compact shoals during daylight hours, and that during darkness they were distributed fairly evenly over the whole area of the tow. The process of dispersal started just before

TABLE I. DESCRIPTION OF CHARTS

Pls. I, II	Times of tow, G.M.T.	Catch per hour (baskets)	Description of traces	Weather and sea	Remarks
Chart (*a*)	13.10–14.55 h, 17. ix. 55	70	Dense 'Thumbprint' recordings throughout the whole tow. Few crescent traces due to single fish	Overcast, fairly heavy swell	Tow made in daylight ending shortly before sunset. The fish are mainly in discrete shoals forming a composite target. Average height of fish from sea-bed 10 fm
Chart (*b*)	15.45–17.45 h, 17. ix. 55	60	Fairly dense traces at the beginning of the recording, thinning out into less distinct single crescent echoes	No moon, overcast, fairly heavy swell	Tow made during and after sunset. The shoals have split up at sunset and the fish have dispersed evenly over the whole of the tow between the sea-bed and 30 fm above
Chart (*c*)	18.30–20.30 h, 17. ix. 55	75	Single crescent echoes throughout the tow	No moon, overcast, fairly heavy swell	Tow made during darkness. The fish are spread out over the whole tow area between the sea-bed and 30 fm above
Chart (*d*)	21.10–23.30 h, 17. ix. 55	50	As (*c*)	As (*c*)	As (*c*)
Chart (*e*)	00.10–02.30 h, 18. ix. 55	50	As (*c*) and (*d*)	As (*c*) and (*d*)	As (*c*) and (*d*)
Chart (*f*)	04.00–06.00 h, 18. ix. 55	35	Dense 'Thumbprint' traces throughout recording with some single crescents	Hazy, fairly heavy swell	Tow made after sunrise. The fish have re-formed into compact shoals; average height of fish from sea-bed 10 fm
Chart (*g*)	06.25–08.15 h, 18. ix. 55	35	As recording (*f*)	Bright and clear, fairly heavy swell	Tow made in daylight. The fish are mainly in discrete shoals as on the previous daylight recordings (Charts (*a*) and (*f*)). Average height of fish from sea-bed 10 fm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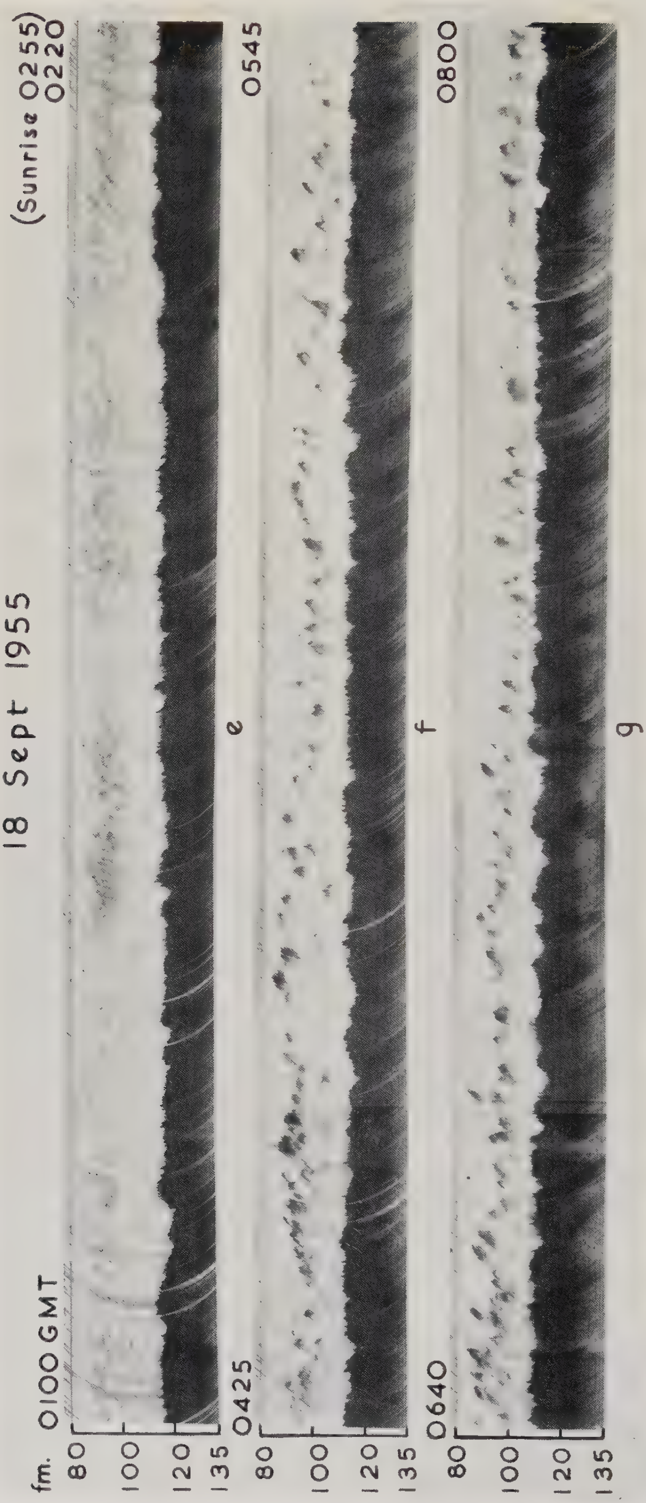
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0800
g

the sunset at 16.12 G.M.T., presumably when the light intensity fell below the threshold of vision of the cod. Conversely, there is evidence to show that the re-forming of the shoals took place at sunrise (02.55 G.M.T.).

The average height of the fish above the sea-bed remained at about the same level (10–15 fm) irrespective of the formation of the fish. Unlike sprats and herring, which have been shown by Richardson (1952) to exhibit diurnal vertical migration, the cod maintained the same average level throughout daylight and darkness, although the maximum height above the sea-bed was greatest in darkness.

The maximum possible vertical movement of the fish on dispersal at night, i.e. 30 fm (about 25% change in level), was well within the theoretical limits imposed by the swim-bladder as deduced by Jones (1952).

The catches did not vary significantly with the change in formation of the fish.

SUMMARY

During a commercial fishing voyage to the Barents Sea continuous observations were made on the shoaling behaviour of cod in deep water over a period of 19 h.

The cod were studied by the use of a Kelvin and Hughes MS. 24J recording echo-sounder.

Compact cod shoals recorded in 100 fm during daylight dispersed at sunset and re-formed at sunrise.

The maximum possible vertical movement of the fish during observation was 30 fm.

The catches did not vary significantly with the formation of the cod.

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THE USE OF TRAWL, GRAB AND CAMERA IN ESTIMATING MARINE BENTHOS

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(With Plates I–IV and Text-fig. 1)

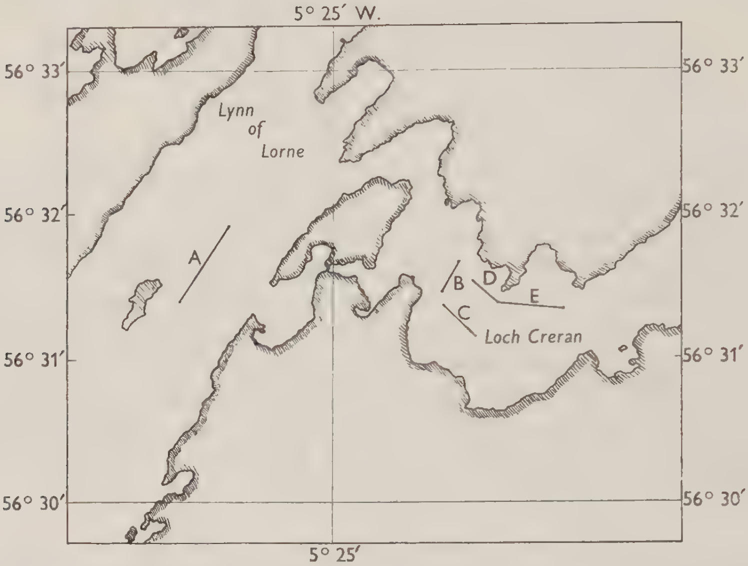
Certain animals of the epifauna, because of their distribution over the bottom, are often difficult to sample quantitatively. They may occur as individuals widely dispersed over a large area, or they may be present in dense aggregations which themselves have a patchy distribution. In the past, workers have tried to estimate the numbers of such animals by the combined use of trawls and grabs of various types. The post-war development of underwater photography suggests that the camera will be a useful additional tool (e.g. Vevers, 1951, 1952). During the testing of an underwater camera from Aberdeen an opportunity was taken to compare the estimates of some of the larger epifauna from grab and trawl hauls with estimates derived from underwater photographs. The results are described in this paper.

THE GEAR

A $\frac{1}{10}$ m² Van Veen type grab, weighted to 72 lb. (33 kg) and fitted with the endless warp rig, was used. The trawl was a standard Agassiz with a 6 ft. (182 cm) beam. The underwater equipment for the camera was designed by Mr R. E. Craig. The unit was a Royal Air Force F-24 camera using $5\frac{1}{2}$ in. aerofilm giving negatives of roughly 13 × 11 cm. Light was provided by an electronic flash apparatus, and a yellow filter was used. Technical details of this equipment are not discussed here.

METHODS

The work was carried out in Loch Creran, and in the Lynn of Lorne, near Oban in Argyllshire. Dans were set out marking five stretches varying in length from 417 to 1174 m. The positions of these stretches are shown in Text-fig. 1. On each stretch a similar procedure was followed. First the ship was allowed to drift between the two dahns while a series of photographs was taken. At the beginning of the run the camera was lowered to the bottom, and was raised a few feet after each exposure to allow the equipment to recharge. Recharging generally took about 30 sec, so that a continuous series of photographs was taken over the ground at a rate of one exposure per minute. The ship was then allowed to drift over the same ground while a series of grab hauls was made. Finally, the Agassiz trawl was towed between the two dans. Since the distance between the dahns was so short, it was fairly certain that between any two sets the three gears covered the same ground, and this was confirmed by an examination of traces from the ship's echo-sounder, which was run continuously.



Text-fig. 1. Chart of the area studied, showing the positions of the five stations. From Admiralty Chart No. 2814A.

All the larger animals of the epifauna caught by trawl and grab were counted and identified. The nomenclature used is that in the *Plymouth Marine Fauna* (Marine Biological Association, 1931). In dealing with photographic data, series of approximately 21 × 17 cm prints were made. Each print represented almost 1 m^2 of bottom, but since the focus often deteriorated towards the edges the prints were masked, and animals on a $\frac{7}{10}$ m^2 area were counted.

RESULTS

Details of the estimates by the three gears are given below for each ground separately. The Agassiz trawl catches are summarized in Table 1.

Stretch A (Pl. I A)

This was in the Lynn of Lorne in 22–28 m. The dans were 1174 m apart, and the ground was covered by seventy-two photographs and twenty-one grab hauls, as well as the Agassiz haul. The total area of bottom considered, i.e. the area swept by the trawl, was 2147 m^2.

Table I. Numbers of Animals taken in each Stretch by the Agassiz Trawl

Animal	Stretch number				
	A	B	C	D	E
Ophiothrix fragilis	1093	1992	215	524	290
Asterias rubens	2	5	2	—	10
Other Asteroidea	3	5	2	—	7
Echinoidea	24	36	20	1	18
Chlamys opercularis	23	4	27	12	118
Lima	—	83	35	8	5
Other Mollusca	—	2	3	2	42
Decapoda reptantia	17	29	20	29	84
Decapoda natantia	—	5	38	38	14
Ascidians	—	45	21	6	14
Fish	8	2	1	4	16

As shown by the photographs, the north end of the ground was of uniform sand, which merged about half-way along the stretch into a short area of coarser sand and gravel. This gave place to mud (Pl. I B) which covered most of the second half of the stretch, with the exception of a few patches here and there where shells, mostly of *Cyprina islandica*, were mixed with the mud. The main animals of the epifauna are considered below.

Ophiothrix fragilis was the most abundant animal encountered, but photographs showed it to be confined to the stretch of sand and gravel, occurring in the mud area only on the shell patches. Even on the hard ground, however, the distribution of *Ophiothrix* was by no means uniform. It occurred on each of the first thirty prints, ranging from one to sixty individuals per frame, with an average of thirty-three. The following nine prints, although over apparently similar ground, showed no *Ophiothrix*, but just before the beginning of the muddy ground they again appeared, with an average of eighteen per print. Knowing the number of photographs taken, it is possible to calculate that the sandy stretch was 650 m long, so that the Agassiz on this part swept an area of 1190 m^2. Eleven of the grab samples came from this area, and all except two contained *Ophiothrix*, the mean number being 1·6 per sample. From the photographs the estimated number of *Ophiothrix* in the sand was 39,542, from grabs, 19,418, and the total number taken in the trawl was 1093. The trawl value may be an overestimate for the sandy area alone, since it is assumed that all the *Ophiothrix* in the trawl were taken on this part.

On this stretch, as well as inside Loch Creran, small numbers of other ophiuroids (e.g. *Ophiocomina nigra* and *Ophiopholis aculeata*) were found mixed with the *Ophiothrix* populations.

Ophiura texturata, although slightly more abundant on the hard ground than on the soft, occurred over the whole stretch, and was seen on fifty-four of the seventy-two prints in numbers ranging from 1 to 7 per print. Single specimens were found in four grab samples, but none was taken by trawl.

From the photographs we can calculate that the total number of *Ophiura* present on the area swept by the trawl was 5325, while the comparable value from the grab data is 4090.

The seventeen kinds of decapoda reptantia found in the trawl consisted of four *Munida bamffica*, one small *Galathea*, nine small spider crabs (mostly *Inachus*) and three *Eupagurus prideauxi*. These same types were also seen on the photographs—a total of eight decapods on seven prints distributed over both the sand and the mud. The grab took one *Eupagurus*. Since the numbers are small it would be misleading to compare estimates for the whole stretch without taking into account the different areas sampled by each gear. The comparison is shown below:

Gear	Area sampled	Estimate for stretch
Grab	2 m²	1074 decapods
Camera	50 m²	341 decapods
Trawl	2147 m²	17 decapods

Twenty-three small specimens of *Chlamys opercularis* were taken in the trawl and none in the grabs. On the photographs only two individuals could be distinguished, but small specimens could easily have been obscured by the overlying brittle-stars. This, together with the fact that a *Chlamys* was usually counted only if both valves could be seen makes it probable that the photographic count is an underestimate.

Among other animals, a flatfish was seen on one print, but no fish were taken by the grabs. In general, however, the fish which the trawl showed to be present in small numbers—codling and *Gadus minutus*—would be difficult to see in photographs. On the soft ground the grab took two *Cyprina islandica* and one *Aphrodite aculeata*. Neither of these was found in the trawl, but although they could not be seen on photographs, numerous tracks were noticed on the mud (e.g. Pl. I B), and many small holes, probably breathing apertures. Lastly, the following echinoderms were 'taken' by the three gears:

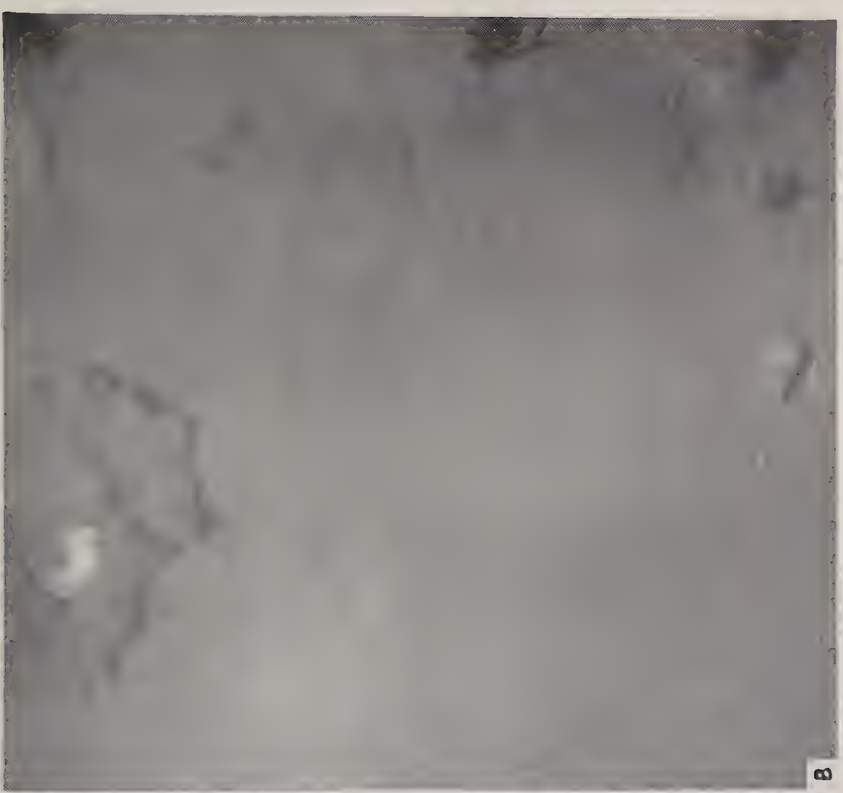
	Totals observed		
	Trawl	Grab	Prints
Echinoids	24	1	4
Solaster papposus	3	0	0
Palmipes membranacea	1	0	0

EXPLANATIONS OF PLATES I AND II

Underwater photographs, each covering approximately ⅔ m² of the sea bottom. Each represents one print used in counting, except that trimming has reduced it by about 5 %. The weight for operating the trigger mechanism can be seen on each print. This was 7 cm in diameter.

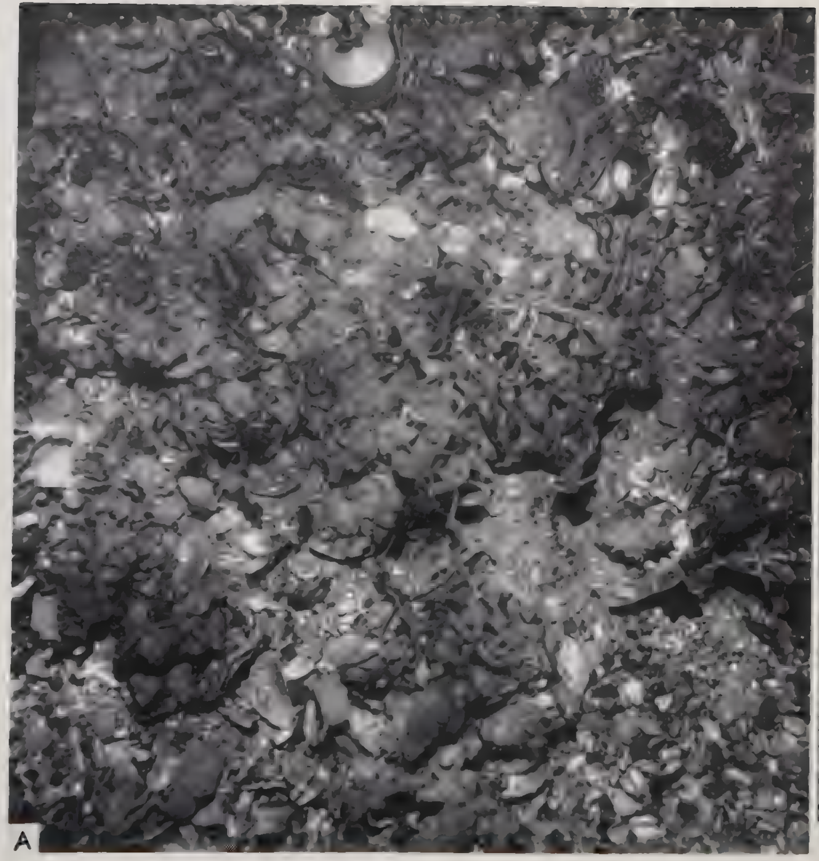
Pl. I. Lynn of Lorne, stretch A: A, showing the sandy area of this stretch and the typical distribution of brittle-stars found in it; B, showing the muddy area, with tracks.

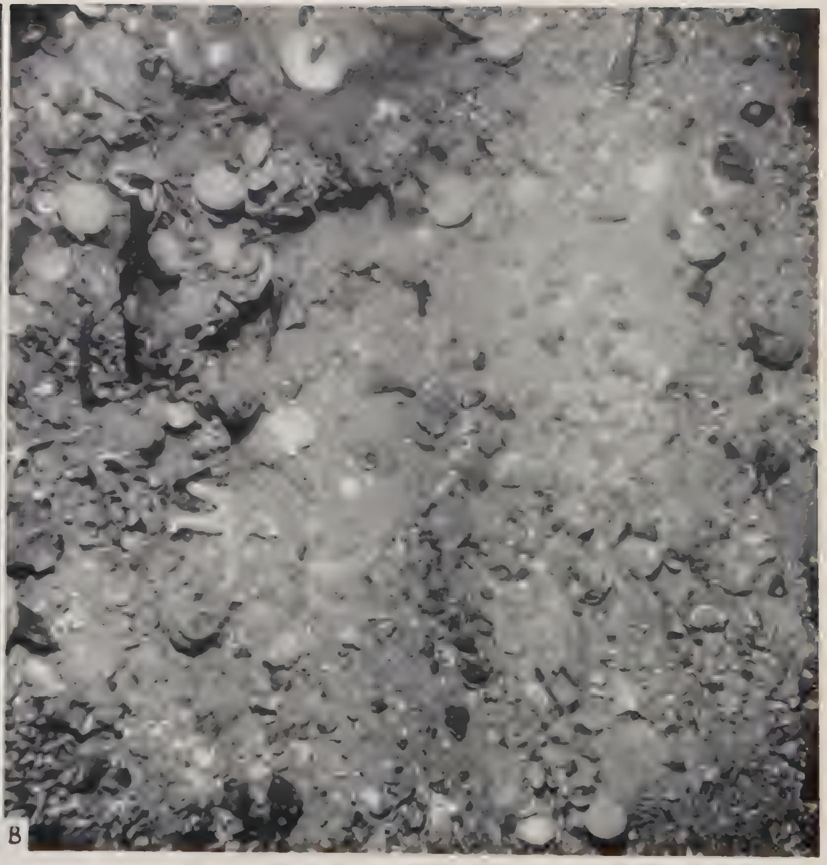
Pl. II. Loch Creran: A, stretch B, showing the epifauna mainly of brittle-stars and ascidians; B, stretch C, coarse deposit where grabbing was difficult, showing *Chlamys opercularis*, starfish and a spider crab.



B

A


A


B

Stretch B (Pl. II A)

This was across the entrance to Loch Creran in 15–22 m. The dans were 464 m apart, and the area swept by the trawl (848 m^2) was covered by sixteen photographs and eighteen grab samples. The photographs showed a fairly uniform stretch of coarse material—sand mixed with stones and shells, with an epifauna of brittle-stars and ascidians.

Ophiothrix fragilis occurred on all the photographs from this stretch, ranging in number from four to seventy-two per print, with a mean of twenty-seven. In the grabs, *Ophiothrix* was found in eleven of the eighteen samples, ranging from one to seventeen per sample, with a mean of 4·9 individuals. The calculated numbers for the whole stretch are 32,702 for the camera and 41,919 for the grab, while the trawl caught 1992.

Lima hians, a lamellibranch which builds a nest among stones and shells, was abundant. Eighty-three were found in the trawl and seven were taken in four grab samples. A nesting animal such as this is difficult to detect on photographs, and only one could be distinguished.

The only natantian decapods found were five *Pandalus* taken in the trawl. The remaining decapods in the trawl consisted of six *Eupagurus bernhardus*, eight *Hyas araneus* and fifteen small spider crabs, mostly *Inachus*. The camera showed four decapods in four prints. Three were spider crabs and the fourth a *Eupagurus*.

Other animals occurring in smaller numbers were as follows:

	Totals observed		
	Trawl	Grab	Prints
Asterias rubens	5	0	8
Henricia	5	1	1
Echinoidea	36	3	0
Holothuroidea	0	1	0
Chlamys opercularis	4	0	6
Buccinum undatum	2	0	2
Lamellibranchs	0	2	0
Goby	2	0	0

Stretch C (Pl. II B)

The dans were placed in 11–28 m depth along the south shore of the loch, and were 579 m apart. The area covered by the Agassiz was 1059 m^2, and was represented by twenty-four photographs and fifteen grabs. The stretch consisted of coarse gravel and stones of various sizes, with some large boulders here and there. Only three of the grab hauls produced fauna—eight *Ophiothrix* three *Lima* and one ascidian. At all the other attempts either the jaws were held open by stones, or the grab was empty, presumably having landed on large boulders. After several further attempts, grab sampling on this stretch was abandoned. On such ground the trawl sampling also is probably far from optimum, and the camera is the one instrument which can operate satisfactorily. The trawl and camera results are considered below.

Ophiothrix fragilis was absent from the first seven prints, although the ground here, except for the presence of a few fragments of algae, appeared to be similar to that of the remainder of the stretch. All the other prints showed *Ophiothrix* in numbers varying from one to forty-two, the mean for all prints being ten. This gives an estimate of 15,577 for the whole area, compared with the trawl catch of 215.

Chlamys opercularis was distributed over the whole area. Twenty-eight individuals were observed on eleven prints in numbers varying from one to ten per print. The estimate for the whole area is 1766 compared with the trawl catch of twenty-seven.

Of the decapods taken in the trawl, thirty-eight were *Pandalus montagui*, and none of these was seen on photographs. Natantian decapods in general probably merge with their surroundings so well that they would be difficult to distinguish on prints. It should be noted that the gear described here is triggered when a weight hits the bottom. The slight interval between the landing of the weight and the taking of the photograph might allow a rapidly moving animal to leave the field. Modification of the camera release would obviate this. The remaining trawl-caught decapods consisted of three *Eupagurus bernhardus*, eleven large *Hyas araneus* and six small spider crabs (*Inachus* and *Macropodia*). Twelve decapods could be seen on ten photographs—one *Eupagurus* and eleven spider crabs, mostly *Hyas*. These spider crabs were distributed fairly evenly over the ground, and the calculated population for the whole area is 694.

Twenty specimens of echinoids were taken in the trawl, and twelve were seen in five photographs. These five photographs were all from a small area at the beginning of the stretch, and on any one print the echinoids usually occurred together and in association with pieces of *Laminaria*.

Other animals are as follows:

	Totals observed			Totals observed	
	Trawl	Camera		Trawl	Camera
Lima	35	—	*Henricia*	2	1
Buccinum	1	2	Sabellids	—	9
Pecten	2	—	Ascidians	21	6
Asterias	2	2	Flatfish	1	—

The nine sabellids all occurred in one small isolated patch of soft ground seen on one print. A number of *Pecten* shells were seen on prints, but since these appeared to be flat on the bottom they were not counted as being living animals.

Stretch D (Pl. III A)

This extended from the entrance along the north shore of Loch Creran, at a depth of 20–28 m. The dans were 417 m apart, giving 763 m^2 as the area trawled, and this area was covered by twenty-six photographs and twenty grabs. The stretch had a fairly uniform bottom of sandy gravel and small stones, with a more or less continuous covering of mussel shells. The most abundant animals were brittle-stars and decapod crustaceans.

Ophiothrix fragilis was fairly evenly dispersed over the ground. It was present on all prints in numbers between twenty-one and seventy-six, with a mean of fifty-six per print. It was also present in all the grab samples, varying from two to thirty-six per sample, with a mean of 10·5. The camera and grab estimates for the whole stretch are 61,330 and 79,686 respectively, compared with 524 individuals taken by the trawl.

Again natantian decapods could not be seen in photographs, although thirty-eight *Pandalus* were found in the trawl and two in the grab samples. The trawl took five large *Hyas araneus* and twenty-one other small spider crabs, and three *Eupagurus*, while three *Hyas* were caught in the grab. Two *Eupagurus* were seen on the photographs and nineteen spider crabs, mostly *Hyas*. The spider crabs were distributed fairly evenly over the stretch occurring on sixteen of the twenty-six prints, giving an estimate of 796 individuals for the whole stretch.

Ascidians were mostly species of *Ascidiella* and *Ciona* and thirty-seven were seen in fifteen prints, in numbers ranging from one to five per print. They were also found in thirteen of the twenty grabs, the total number being twenty-three. Estimates for the whole area amount to 1550 individuals for the camera, and 8769 for the grab. The trawl took six specimens.

Thirty-one *Lima hians* were taken in thirteen grab samples, ranging from one to five per sample. Only eight specimens were taken in the trawl. Trawl catches of *Lima* are likely to be very variable, depending on the extent to which the trawl bites into the bottom. No *Lima* were seen on the prints.

Seven mussels were taken in the trawl and thirteen in nine grabs. On the photographs almost every print showed mussel shells, and in several prints two closed valves could be seen. These lay singly or in small groups, not in a continuous bed. It was, however, difficult to decide if these animals were alive, and mussels were therefore not counted.

The following other animals were found in the three gears:

	Totals observed		
	Trawl	Grab	Prints
Chlamys	12	2	—
Buccinum	1	1	2
Echinoids	1	5	4
Asterias rubens	—	4	8
Solaster	—	—	1
Fish	4	5	1

Stretch E (Pl. III B)

This was a continuation of stretch D along the north shore of the loch in 26–31 m. The distance between dans was 834 m, and the area swept by the trawl 1525 m^2. This area was covered by fifty-seven prints and twenty grabs. Two distinct types of bottom were encountered. About a third of the stretch consisted of sandy gravel with large stones here and there. This merged into mud which covered the remainder of the stretch, except for a few small patches of muddy sand. The prints showed that most of the epifauna occurred on the gravel.

Ophiothrix fragilis occurred only on the area of sand and gravel, which was covered by the first twenty prints, and the numbers were lower than on any of the other stretches. They were seen on fifteen of the twenty prints in numbers varying from two to forty per print, with a mean of eight. Five of the grabs were on the sandy area, and took a total of twelve *Ophiothrix*. The estimates for the whole gravel area for camera and grab are 4916 and 10,455 respectively. The trawl took 290 *Ophiothrix*.

Chlamys opercularis was distributed over the whole area but was more numerous on the gravel than on the mud. No *Chlamys* were taken by the grab. Photographs showed twenty-five individuals in fifteen prints, varying in number from one to four per print, and giving an estimate for the whole area of 956 *Chlamys*. The number taken by the trawl was 118.

Eleven *Pandalus* and three *Crangon* were taken by the trawl, but no natantian decapods were recorded from grabs or photographs. The Reptantia, however, were numerous. Forty-eight spider crabs, mostly small, thirty-five eupagurids and one *Munida* were taken in the trawl. In the grab samples only one *Eupagurus* and one *Galathea* were taken. Twelve crabs were noted in ten prints—five spider crabs and seven *Eupagurus*. The spider crabs were mostly large *Hyas*, and the population of small spider crabs sampled by the trawl could not be estimated from photographs.

Other animals which occurred in smaller numbers were as follows:

	Totals observed		
	Trawl	Grabs	Prints
Pennatulacea	—	—	1
Pecten	1	—	2
Mussel	13	—	+
Cockle	2	1	—
Asterias rubens	10	—	1
Solaster endica	3	—	1
Solaster papposus	2	—	4
Henricia	1	—	1
Palmipes	1	—	—
Ascidians	—	8	3
Fish	16	—	1

The ascidians in the grabs were taken in two clumps in two consecutive hauls, and those in photographs were on consecutive prints, suggesting that these animals were localized in a small part of the stretch, which could account for their absence from the trawl. The fish consisted mainly of gobies and flatfish.

DISCUSSION

In considering three gears as different as trawl, grab and camera it is obvious that direct comparisons cannot be made. Each gear has its own peculiarities which give it advantages and limitations not possessed by the others. In dealing with the epifauna one of the important factors is the area of bottom which can be conveniently sampled. Thus between two dans in the present work, the trawl in half an hour could cover 2000 m^2 of bottom, the camera in the same time photographed 50 m^2 and the grab in half an hour generally sampled about 2 m^2.

Since the trawl covers a large area it is useful in sampling organisms which are few in number and widely dispersed. Further, it can take large animals, and also active ones such as natantian decapods. But the trawl gives no indication of the distribution of the animals within the limits of the comparatively extensive ground covered in any one haul. A series of grab samples can give information on distribution, but since each sample covers so small an area, an impossibly large number of samples would be required before grab results for less numerous animals could be applied to a wide area. The camera is intermediate between the trawl and grab in that it can cover a fairly wide area yet indicate in considerable detail the distribution of the animals. On stretch C, for example, the trawl showed merely the presence of *Chlamys opercularis* and of Echinoidea. The camera, however, established that while the former species was distributed fairly evenly over the whole stretch, the latter group was confined to a small area, and even there occurred only in association with *Laminaria*.

In practice, the relative effectiveness of the three gears will vary with the numerical density of any particular species, and with its distribution over the whole area studied. The most abundant animal in the present work was *Ophiothrix fragilis*, and the various estimates of the numbers of this species on the five stretches are given in Table II.

TABLE II. NUMBERS OF *O. FRAGILIS* PRESENT ON EACH STRETCH AS ESTIMATED BY THE THREE GEARS

Stretch	Trawl	Camera	Grab
A	1093	39,542	19,418
B	1992	32,702	41,919
C	215	15,577	(No samples)
D	524	61,330	79,686
E	290	4,916	10,455

The trawl always gave the lowest estimate. The camera values are from 16 to 117 times greater, and the grab values from 18 to 152 times greater than those of the trawl. Comparing the camera and the grab, it can be seen that on stretch A the camera estimate is higher, while on the other four stretches the grab gives a rather greater value. The difference between stretch A and the other grounds with regard to the relative estimates of camera and grab is connected with a difference in the distribution of the animals (cf. Pls. I A and IV). In the Lynn of Lorne generally (where stretch A is located), the brittle-stars are found not in dense masses but either as single individuals (Pl. I A), or in small clumps of up to five or six individuals. With such a distribution there are large patches of bare ground and the grab will tend to underestimate the number present. The camera, on the other hand, covering a larger area, gives a better estimate. In Loch Creran, however, the brittle-stars occurred in dense masses (Pl. IV) or at least as a fairly even covering over the ground (Pl. III A). For such distributions the grab gave rather higher estimates than the camera, probably partly because in counting brittle-stars in prints the general practice was to count an animal only when the disc could be seen. Thus when the animals are aggregated, often several individuals thick, the discs of animals in the lower layers tend to be obscured and an underestimate results. It should be noted, however, that although in these conditions the camera may give a slightly lower estimate than the grab, it is a much less variable, and therefore more reliable estimate. On stretch D, for example, where the densest population of brittle-stars was found, the coefficient of variation of the grab counts was 85 %, while the corresponding value for the camera was only 25 %.

The other animals of the epifauna were all considerably less abundant than *Ophiothrix*, and usually occurred as single individuals often widely separated from each other. For animals distributed in this way the grab is generally the least efficient of the three gears, since, covering such a small area, it takes too few individuals to allow valid estimates to be made for the whole ground. The trawl is better, mainly because it covers considerably more ground. The best results were usually from the camera. For example, in stretch D, only three of the twenty grab samples contained spider crabs, while sixteen of the twenty-six photographs showed these animals, giving an estimate of 796 individuals for the whole area, compared with the trawl catch of twenty-six. Another species on which the camera gave useful information was *Chlamys opercularis*, and the estimated numbers of this species on each stretch are shown in Table III. The camera not only most often gave higher estimates, but also gave information not obtained from the other gears. It showed on stretch E, for example, that *Chlamys* was distributed over the whole stretch; that it was more abundant on the gravel than on the mud, and that its density varied from one to four individuals per square metre.

EXPLANATION OF PLATES III AND IV

Underwater photographs, those on Pl. III covering approximately $\frac{2}{3}$ m^2 of the sea bottom, as in Pls. I and II; that on Pl. IV about $\frac{7}{10}$ m^2. The weight for operating the trigger mechanism, seen on each print, was 7 cm in diameter.

Pl. III. Loch Creran: A, stretch D, showing the covering of mussel shells; B, stretch E, showing the coarse deposit with *Chlamys opercularis*.

Pl. IV. Example of the aggregation of brittle-stars found on several stretches in Loch Creran.

A

B

(*Facing p.* 428)

TABLE III. NUMBERS OF *CHLAMYS OPERCULARIS* ON EACH STRETCH AS ESTIMATED BY THE THREE GEARS

Stretch	Trawl	Camera	Grab
A	23	85	0
B	4	454	0
C	27	1766	No samples
D	12	0	763
E	118	956	0

Finally, it should be noted that the use of the camera is limited to areas of fairly clear water. It is difficult to obtain clear pictures, for example, in the turbid water of Aberdeen Bay, and off some parts of the south-west Scottish coast.

SUMMARY

Agassiz trawl and Van Veen grab catches of certain animals of the epifauna are compared with numbers of these animals estimated from underwater photographs.

In sampling brittle-stars, which occurred in large numbers, the Agassiz trawl was the least efficient of the three gears. When the brittle-stars were distributed singly or in small patches the camera gave better results than the grab. When the patches were large, or when the distribution was more or less continuous, the grab gave slightly higher estimates, but its estimates were considerably more variable than those of the camera.

For the less common animals of the epifauna the grab was generally a poor sampling instrument. The trawl was rather better, but within fairly wide limits it can give no indication of distribution. The camera gave more acceptable estimates and also indicated the distribution of the animals over the ground.

For certain areas of hard bottom the camera is probably the only instrument which can give adequate results.

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THE ATTENUATION OF LIGHT IN SEA AND ESTUARINE WATERS IN RELATION TO THE CONCENTRATION OF SUSPENDED SOLID MATTER

By D. Jones and M. S. Wills
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(Text-figs. 1–8)

Experiments have been reported recently (Atkins, Jenkins & Warren, 1954) in which the relative concentration of suspended matter at different depths in the sea was determined by filtering samples of water through collodion membranes and measuring the relative albedos. Also reported in the same paper are the results of a series of observations of the visual range of a Secchi disc at station E 1 in relation to the concentration of phytoplankton.

Experiments are now described which were designed to investigate the relation between the Secchi disc reading, the concentration of suspended matter, and the attenuation coefficient of tungsten light in sea water.

Thanks are due to the Admiralty for permission to publish this paper, and to Mrs L. M. Lewis of A.R.L. who assisted with the experimental work. The authors are also very grateful for assistance and facilities provided by the Marine Biological Association of the U.K. and by the Water Pollution Research Laboratory of D.S.I.R.

MEASUREMENT OF ATTENUATION COEFFICIENT

It is well known that the optical properties of a sample of natural water may be affected during collection and removal to a laboratory (Hulbert, 1945; Jenkins & Bowen, 1946; Jerlov, 1951). Such measurements should therefore be made as far as possible *in situ*. The Admiralty Research Laboratory hydrophotometer is an instrument which may be lowered into water at any desired position, and measures the attenuation of an approximately parallel beam of light from a tungsten filament lamp traversing a path of length 50 cm. in the water. It is somewhat similar to that developed by Pettersson (1934), the main difference being that the A.R.L. hydrophotometer has been designed to prevent as far as possible scattered light falling on the photocell. The instrument consists of a lamp-housing and a photocell-housing rigidly connected together by three tubes and may be seen in the photograph of Fig. 1. A diagram of the optical system and the electrical circuit is given in Fig. 2. Light from the bulb *B* is rendered parallel by the lens L_1 and passes through the $\frac{3}{8}$ in. plate glass window W_1 into the turbid water *T*. After traversing a path

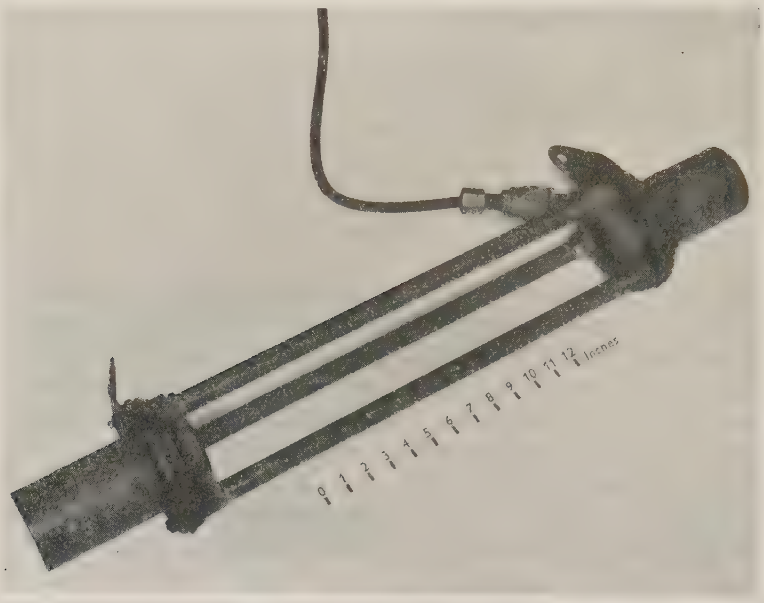
0
1
2
3
4
5
6
7
8
9
10
11
12
Inches

Fig. 1. The A.R.L. hydrophotometer.

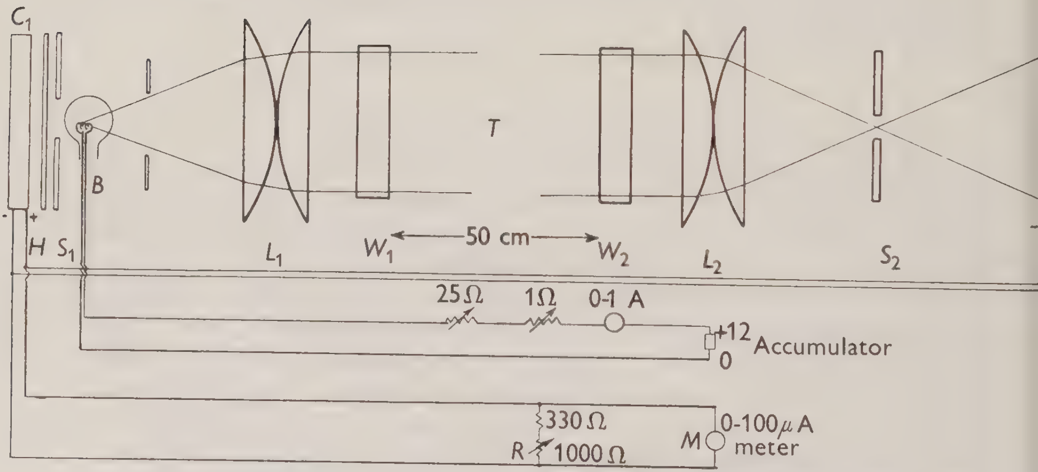
C_1
T
B
-
+
H
S_1
L_1
W_1
50 cm
W_2
L_2
S_2
25Ω
1Ω
0-1 A
+12
0
Accumulator
330Ω
R
1000Ω
M
0-100µA
meter

Fig. 2. Optical system and electrical circuit of A.R.L. hydrophotometer. For explanation see text.

of length 50 cm. the light enters the photocell-housing through the window W_2, and is brought to a focus near the plane of the stop S_2. Although the image is formed in a slightly different position according to whether the instrument is in water or in air, all the light still passes through S_2. It finally falls on a Weston barrier layer photocell C_2 which incorporates a green filter producing a spectral response approximating to that of the eye. The stop S_2 effectively prevents daylight reaching the photocell C_2, and so the instrument can be used equally well in daylight. A similar photocell C_1 is located inside the lamp-housing and a portion of the light from B passes through the adjustable stop S_1 and falls on C_1. A heat-absorbing glass H is necessary to minimize heating of C_1 by the lamp with consequent change of sensitivity. The two cells C_1 and C_2 are connected in opposition as shown. In the laboratory, with the instrument in air, S_1 is adjusted so that the outputs from the two cells balance. This method of design compensates for variations in intensity of the light source. Just before the instrument is used in water the light-beam is blocked from C_2 and the meter M is set to full-scale deflexion (100 divisions) by means of the variable resistance R. When the instrument is immersed in water, the light transmitted increases by a factor of approximately 1·08 since two air-glass reflexions are eliminated. Thus the meter reading obtained when the instrument is immersed has to be corrected, and the percentage light transmission per half metre t is given in terms of the meter reading M by the formula

$$t=(100-M)\,0{\cdot}92. \tag{1}$$

It is well established that the absorption and scattering of light in a turbid medium obey an exponential law, and thus an attenuation coefficient μ (metre)$^{-1}$ can be introduced given by the formula

$$t=100\exp(-0{\cdot}5\mu). \tag{2}$$

It should be emphasized that this coefficient is not the same as the vertical extinction coefficient (μ_v) as found from the variation with depth in the sea of the daylight falling on an upturned horizontal surface which is defined (Poole & Atkins, 1928) by

$$\mu_v=\frac{2{\cdot}3}{d}\{\log_{10} V_1-\log_{10} V_2\}, \tag{3}$$

where V_1 and V_2 are the simultaneous values of the illumination at two points differing in depth by d metres. This is because illumination on a horizontal surface at any depth in water is partly due to light that has been scattered by the water, whereas the attenuation coefficient refers only to a parallel beam (the instrument being designed to eliminate as far as possible the effect of scattered light). It should be mentioned, however, that owing to certain unavoidable limitations a little forward scattered light will always be collected by such an instrument; the error introduced as a consequence of this factor is discussed in the Appendix to this paper.

The current generated by a barrier-layer photocell is no longer proportional to the light intensity when the resistance in series with the cell is too high. It was therefore necessary to test the linearity of response of the instrument. This was done by introducing into the beam (in air) a number of thin glass plates, and measuring the change in galvanometer deflexion as each plate was added. Allowance was made for multiple reflexions between the surfaces of the plates when calculating the attenuation produced by a given number of plates. With the electrical circuit used the response was accurately linear.

MEASUREMENTS WITH SUSPENSIONS MADE UP IN THE LABORATORY

Preliminary experiments were carried out in the laboratory using suspensions of kaolin or Thames mud in tap water. The hydrophotometer was placed in a tank containing about 15 l. of water. Known amounts of a concentrated suspension of kaolin (1000 p.p.m.) or Thames mud (200 p.p.m.) were stirred into the tank and the light transmission was measured at each value. In Fig. 3 is shown a graph of the attenuation coefficient per metre (μ) plotted against the concentration in mg/l. for the kaolin and Thames-mud suspensions.

EXPERIMENTS AT SEA

Method of Obtaining Samples and Measurements

In the measurements on the water at sea one end of a length of clean $\frac{1}{2}$ in. rubber hose pipe was lashed to the hydrophotometer which was then lowered into the sea from a boat. The other end of the pipe was connected to a carefully cleaned 2 l. glass bottle which could be evacuated by means of an electrically driven pump running from a 24 V accumulator. Water was drawn up the pipe into the sample bottle and at the same time the light transmission was measured. The bottle was filled and emptied overboard at least three times before the final sample was taken.

Measurements were made at various depths down to 12 m, and the Secchi disc visibility was also observed using a white disc of 20 cm diameter. The observations were made at various positions in the Thames Estuary between Gravesend and the Nore, and in the sea and estuarine waters near Plymouth. Altogether thirty-four samples of water were filtered, covering a range of light transmission from 5 to 90% per half metre. Details of the observations are given in the table of results (Table I), including light transmission measurements for some positions where no water sample was taken. The positions of the stations are shown on the maps in Figs. 4 and 5.

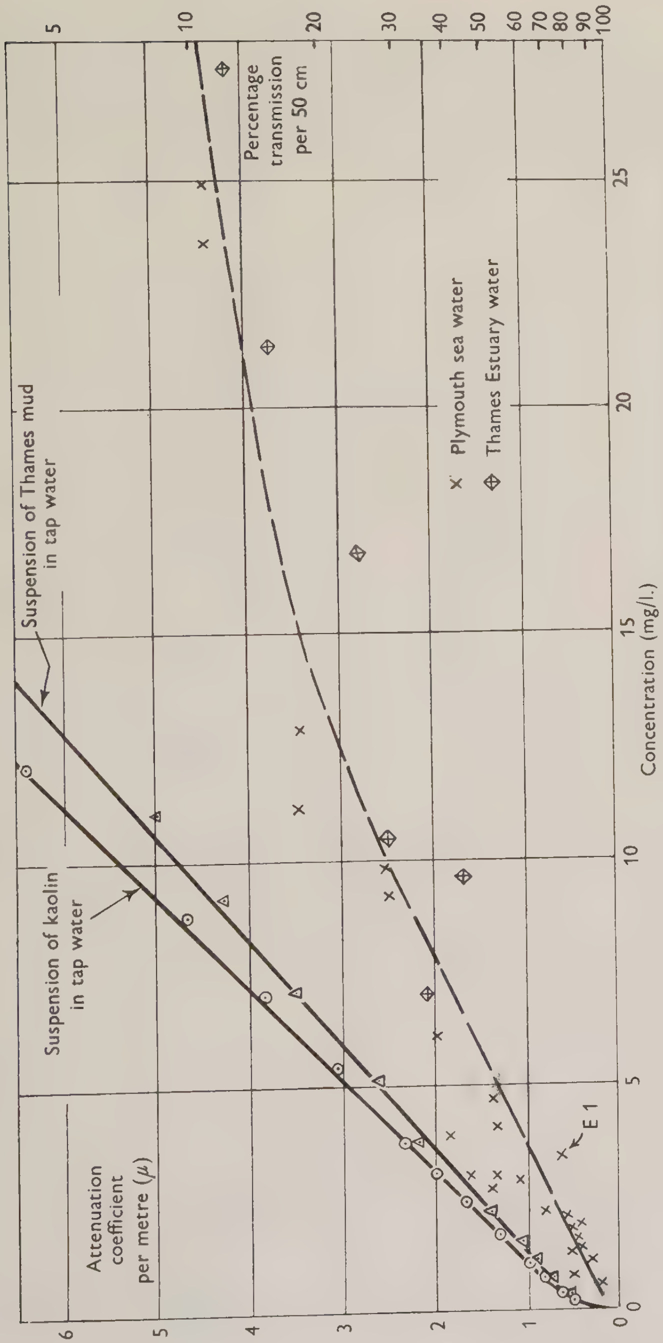

Fig. 3. Relation between attenuation coefficient per metre and concentration of suspended solid matter.

TABLE I. RESULTS

AHW, after high water; BHW, before high water; *c*, concentration in mg/l.; *D*, Secchi disc visibility (metres); *S*, salinity in g/kg; *w*, ash weight in mg/l.; μ, attenuation coefficient per metre; $\bar{\mu}$, average attenuation coefficient between surface and depth *D*; *, indicates two independent determinations of weight of suspended solid. Only the observed values, μ and $\bar{\mu}$ are given in the table, and the correction discussed in the Appendix has not been made. Thus the values are likely to be up to 30 % too small.

Date	Station	Time	Depth (m)	μ	*S*	*c*	*w*	*D*	$\frac{1}{D}$	$\bar{\mu}$
6. v. 53	A	1½ h BHW	0·3	0·278	34·8	1·1	—	21	.048	0·211
			9·0	0·188	34·5	0·6	—	—		—
	B	1¼ h BHW	0·3	0·472	34·0	1·8	—	15		0·304
	C	½ h BHW	—	—	—	—	—	8		0·56
	D	¼ h BHW	0·3	0·80	32·7	2·2	—	6·5		0·68
	E	HW	—	—	—	—	—	11		0·445
	F	2 h AHW	—	—	—	—	—	5		0·89
	G	2¼ h AHW	0·3	1·35	33·6	2·7*	0·99*	3		1·34
						4·7	1·25			
	H	2¾ h AHW	—	—	—	—	—	10·5		0·472
	I	3½ h AHW	0·3	1·83	28·6	3·9	1·4	2·5		1·70
	J	4 h AHW	4·5	1·60	30·6	3·0	0·9	2		1·80
	K	4¼ h AHW	10·5	1·06	32·1	2·9	—	3		1·54
	L	5 h AHW	3·0	0·522	34·0	2·1	0·6	9		0·48
	M	5 h AHW	12·0	0·446	34·7	1·6*	0·5	5		0·92
						1·9				
11. v. 53	E1	5 h BHW	12·0	0·576	—	3·5	0·3	7		0·535
13. v. 53	N	3¾ h AHW	9·0	0·472	34·8	1·9*	—	8·5		0·554
						1·8				
	I	5 h AHW	9·0	4·42	31·0	23·6*	—	1		5·20
						24·9				
	P	5¼ h AHW	6·0	3·44	32·1	12·9*	—	1·5		3·63
						11·1				
	Q	5½ h AHW	12·0	2·48	33·1	9·7*	—	1·75		2·50
						9·2				
	C	5¾ h AHW	6·0	1·94	33·6	6·1	—	2·25		2·19
	R	6 h AHW	3·0	1·31	33·8	3·0*	—	3		1·31
						4·1				
	N	4 h BHW	1·5	0·80	34·5	1·5*	—	6		0·80
						1·4				
	S	3¾ h BHW	6·0	0·498	35·1	1·3*	—	8		0·498
						0·8				
18. v. 53	S	½ h AHW	—	—	—	—	—	6		0·67
	N	¾ h AHW	—	—	—	—	—	5		0·71
	M	1¾ h AHW	—	—	—	—	—	4		0·97
19. v. 53	C	3½ h AHW	—	—	—	—	—	2·5		2·26
	T	4 h AHW	—	—	—	—	—	1·5		2·97
	P	4¼ h AHW	—	—	—	—	—	1·33		4·60
	I	4½ h AHW	—	—	—	—	—	0·75		6·41
9. vii. 53	TA	1¼ h AHW	3·0	1·69	—	9·6	—	—		—
	TB	1½ h AHW	3·0	2·04	—	7·0	—	—		—
			9·0	2·48		10·4				
	TC	2 h AHW	7·5	2·70	—	16·7	—	—		—
	TD	3 h AHW	3·0	4·2	—	27·3	—	—		—
	TE	3½ h AHW	6·0	3·7	—	21·2	—	—		—

Determination of Suspended Solid Matter

The water samples were taken to the laboratory and filtered within 48 h through 'Gradocol' nitro-cellulose membranes, as described by Armstrong & Atkins (1950), of average pore diameter 1·3 to 1·6 microns, using an Elford filtering equipment. The samples were kept in the dark before being filtered to prevent the growth of algae. One of the filtrates was filtered a second time through a 0·6 micron membrane. Less than 0·05 mg was obtained from 2 l. of filtrate, indicating that the weight of solid matter passing through the 1·6 micron pores was negligible.

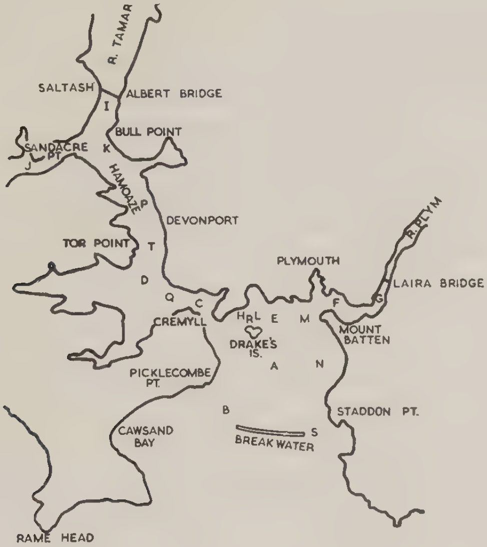
R. TAMAR
SALTASH
ALBERT BRIDGE
I
BULL POINT
SANDACRE PT.
K
J
HAMOAZE
P
DEVONPORT
TOR POINT
T
D
PLYMOUTH
R. PLYM
LAIRA BRIDGE
Q
C
CREMYLL
H
R
L
E
M
F
G
MOUNT BATTEN
DRAKE'S IS.
A
N
PICKLECOMBE PT.
B
STADDON PT.
CAWSAND BAY
BREAKWATER
S
RAME HEAD

Fig. 4. Plymouth Sound and Hamoaze.

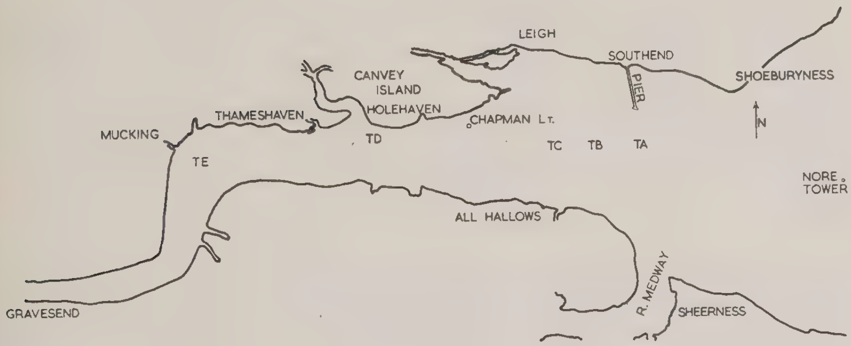
LEIGH
SOUTHEND
SHOEBURYNESS
CANVEY ISLAND
PIER
HOLEHAVEN
THAMESHAVEN
CHAPMAN LT.
N
MUCKING
TD
TC
TB
TA
TE
NORE TOWER
ALL HALLOWS
R. MEDWAY
SHEERNESS
GRAVESEND

Fig. 5. Mouth of the River Thames.

The technique used to determine the weight of dry solid matter was as follows. The membranes which were supplied immersed in water were first dried to constant weight (a) on porcelain dishes at 50° C. According to the Wright-Fleming Institute this does not materially alter the pore size of the membranes used. A measured volume of 1 or 2 l. of the water was then filtered through the membrane under a reduced pressure of about 15 in. of mercury. A portion of the filtrate was used to remove the last traces of solid matter from the sample bottle and filtering apparatus. The membrane was weighed wet (b), and then dried on the same dish at 50° C, and finally it was weighed again (c). The wet weighing was needed in connexion with the method of allowing for the small amount of sea salts remaining in the membrane after drying. It was considered safer to determine this weight accurately rather than to try and wash away the salts with distilled water. To make this correction a known weight of the filtrate (d) was evaporated at 50° C and the weight of residual salt determined (e). In some of the later determinations a clean dry membrane of the same kind was soaked in the filtrate and dried, so as to make sure that the drying conditions with and without the suspended matter were identical. Let the weight of salt present with the suspended matter $=x$. The loss of weight on drying was in each case proportional to the weight of salt present and hence $x/(b-c)=e/(d-e)$. Thus the salt correction x was calculated and the corrected weight of suspended matter was $c-a-x$. In addition to correcting for the salts this method also allows for any water combined with the salts which was not driven off at 50° C.

Some ash weight determinations were also made by the following method. After being weighed dry the membrane was put back into the filtering apparatus and washed with a filtered dilute solution (approximately N/2) of ammonium nitrate to dissolve out the salts. It was then transferred to a weighed platinum crucible and ignited in a muffle at 550° C for 30 min, and finally cooled in a desiccator and weighed. The ash weight of the membrane itself was negligible (less than 0·05 mg). The ash weights are also given in Table I.

Discussion of Results

Relation between the Attenuation Coefficient and the Concentration of Suspended Matter

In Fig. 3 the experimental results are shown plotted in the form of the attenuation coefficient μ against the concentration of suspended matter in mg/l., approximately equal to parts per million (p.p.m.). The attenuation coefficient was deduced from the hydrophotometer readings by the use of equation (2).

For the suspensions of kaolin and mud in tap water the attenuation is seen to be a linear function of concentration apart from a slight unexplained curvature near the origin. For sea water the points are much more scattered, as might be expected in view of the variations in the nature of the suspended material from place to place, and the experimental errors involved in filtering and weighing. The curve in the figure has been drawn so as to represent, as nearly as possible, the average properties of the different samples investigated. It is clear from the results that the attenuation coefficient is approximately proportional to the concentration only for the lower concentrations and rises less steeply than for the freshwater suspensions, and less and less steeply as the turbidity increases. These results may doubtless be attributed to variations in the nature or particle size distribution of the suspended material. As regards the effect of variations of particle size, the total projected area of the particles for a given amount of material decreases as the particle size increases, i.e. as flocculation proceeds. It is to be expected that the suspended matter present in salt water is in a more flocculated state than suspensions made up in fresh water.

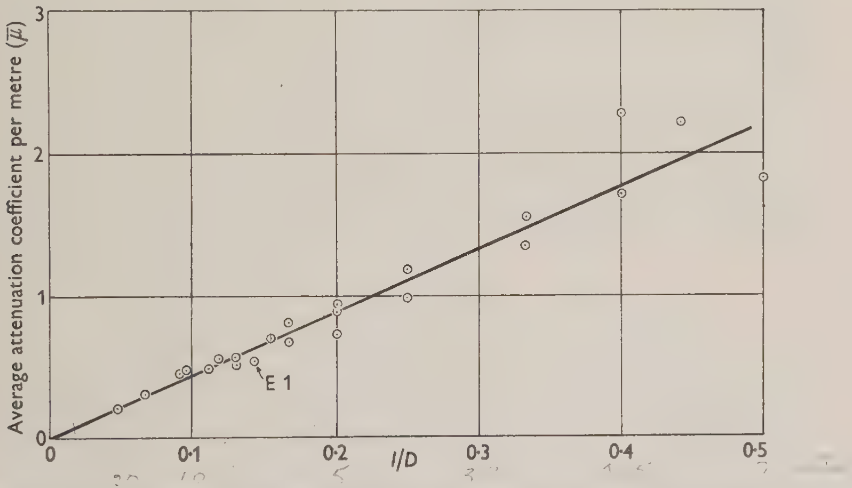

Fig. 6. Relation between attenuation coefficient per metre and Secchi disc visibility.

The point marked E 1 in Fig. 3 was for an observation made at Hydrographic Station E 1, *ca.* 10 miles south-west of the Eddystone, when there happened to be an exceptionally large growth of plankton containing much *Phaeocystis*. The light transmission was 75% per half metre, and the concentration of dry matter came out to be 3·5 mg/l. The usual concentration for this value of light transmission was about 2 mg/l. near the land in the neighbourhood of Plymouth. The weight of the E 1 material after ignition was only 0·3 mg/l., showing that little material apart from the organic matter was present, as would be expected so far from land.

Relation between the Attenuation Coefficient and Visibility of a Secchi Disc

For the positions where the Secchi disc reading D metres was observed the mean attenuation coefficient $\bar{\mu}$ between the sea surface and depth D was calculated. The results covered the range $D=21$ m to 0·75 m, corresponding to $\bar{\mu}=0{\cdot}2\ \mathrm{m}^{-1}$ to $6{\cdot}4\ \mathrm{m}^{-1}$ and the best relation to fit them all was found to be $\bar{\mu}=(4{\cdot}75\pm0{\cdot}08)/D^{(1{\cdot}04\pm0{\cdot}02)}$. If the few results for the very turbid water ($\bar{\mu}>3$) were excluded, a satisfactory agreement was obtained with the simple formula $\bar{\mu}D=K$ (a constant) with the value $K=4{\cdot}38\pm0{\cdot}1$. Fig. 6 shows $\bar{\mu}$ plotted against $1/D$ together with the straight line $\bar{\mu}=4{\cdot}38/D$.

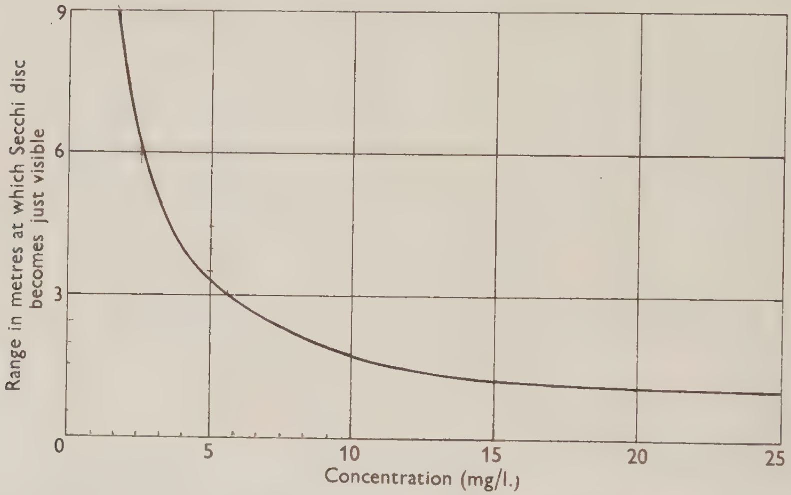

Fig. 7. Relation between Secchi disc visibility and the concentration of suspended matter.

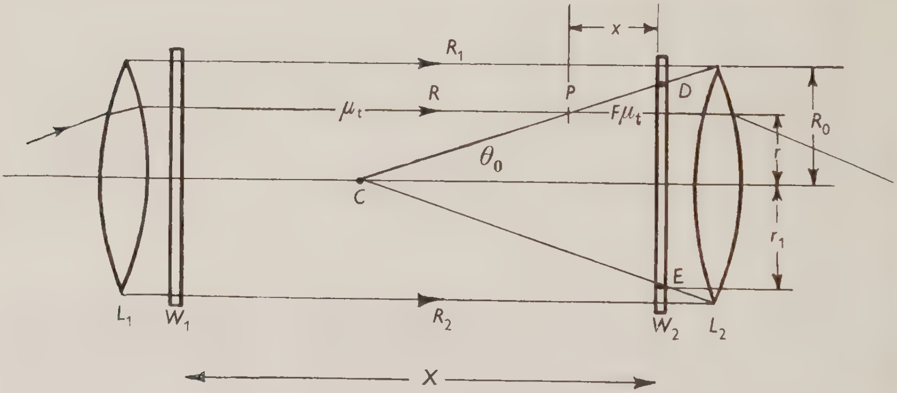

Fig. 8. Limiting cases of collected scattered light.

For Station E1, where the turbidity was mainly due to plankton, the observed Secchi visibility was somewhat less than that predicted from a hydrophotometer reading using the above formula. The actual Secchi disc reading was 7 m, whereas the value deduced from the hydrophotometer reading was 8 m. However, this difference may not be significant in view of the rather large experimental errors associated with a single observation.

In Fig. 7 is shown the relation between Secchi disc visibility and the concentration of suspended matter, deduced by combining the results of Figs. 3 and 6.

SUMMARY

For suspensions of kaolin or Thames mud in fresh water the attenuation coefficient per metre of an approximately parallel beam of light from a tungsten filament lamp was found to be a linear function of the concentration of suspended matter. It reached the value 5 m^{-1} for approximately 10 mg/l. of suspended matter. For sea and estuarine waters near Plymouth and in the Thames Estuary the attenuation coefficient, measured *in situ*, was only about 2·5 m^{-1} for 10 mg/l. and it increased still less rapidly for higher concentrations, reaching only 4·5 m^{-1} at about 28 mg/l. The concentration of suspended matter was determined by filtering water samples through 'Gradocol' membranes, drying the membranes at 50° C, and correcting for the quantity of inorganic salts present with the suspended matter.

The Secchi disc visibility was inversely proportional to the attenuation coefficient over the range 1·5 to 21 metres.

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APPENDIX

The Effect of Forward Scattered Light on the Readings of a Light Transmission Meter

This appendix refers to any light transmission meter having an optical system similar to that shown in Fig. 2 (such as the A.R.L. hydrophotometer).

Owing to the finite size of the stop S_2 which cannot be reduced without loss of sensitivity a certain amount of light scattered by the particles of the turbid medium in forward directions (i.e. in nearly the same direction as the incident light) reaches the detector, and thus the measured light transmission is higher than it would be if the hole were negligibly small.

An accurate theoretical calculation of the effect for a given instrument would involve a long computation for each particle size and would have very little value in practice as a method of correcting the reading because the particle size distribution in the turbid medium is very rarely known. It is, however, desirable to know the order of magnitude of the error, and it is now shown how to determine limits within which the error lies for a given particle size. An experimental determination of the effect in a given turbid medium would involve measuring the angular distribution of the scattered light.

Angular Distribution of the Scattered Light

It can readily be shown that if the lens L_2 has no stopping effect the effective semi-angle subtended by the photocell C_2 is the same at all points in the absorbing medium, equal to $\theta_0 = s/f$, where s is the radius of the stop S_2 and f the focal length of the lens L_2.

It has been pointed out by Walton (1947) that the angular distribution of the scattered light may be calculated by the theory of Fraunhofer diffraction and without recourse to electromagnetic theory for the case of opaque particles which are large compared with the wave-length. According to Walton, if the incident parallel beam is of intensity 1 lumen/cm^2 and an opaque particle of radius a (where a is small compared with the diameter of lens L_2 but large compared with the wave-length λ) is placed in front of lens L_2 it will intercept a quantity of light πa^2 lumens and also diffract a quantity of light which will be distributed over the area of S_2. The total diffracted light is shown to be also equal to πa^2 and the fraction of this lying within a circle of radius s is

$$1 - J_0^2(2\pi as/f\lambda) - J_1^2(2\pi as/f\lambda),$$

or

$$1 - J_0^2(z_0) - J_1^2(z_0),$$

where

$$z_0 = 2\pi a\theta_0/\lambda \quad \text{since} \quad \theta_0 = s/f,$$

where J_0 is the Bessel function of zero order, and J_1 is the Bessel function of the first order.

The parameter which expresses the proportion of scattered light collected is the ratio per particle:

$$\frac{\text{light intercepted} + \text{light scattered outside the cone of semi-angle } \theta_0}{\text{light intercepted} + \text{total light scattered}}.$$

This will be called the factor of merit F. Thus for opaque particles

$$F = \frac{\pi a^2 + \pi a^2\{J_1^2(z_0) + J_0^2(z_0)\}}{2\pi a^2}$$

$$= \tfrac{1}{2}\{1 + J_1^2(z_0) + J_0^2(z_0)\}. \qquad \text{(A 1)}$$

It is clear that for a perfect instrument ($\theta_0 = 0$) in which none of the scattered light is collected, $F = 1$. If some of the scattered light is collected F lies between 1 and 0·5 for opaque particles and may even fall below 0·5 for translucent particles.

Finite Aperture of Lens L_2

The effect of the finite aperture of the lens L_2 will now be considered. If a scattering particle lies near the edge of the beam, or far enough in front of lens L_2, part of the scattered light within the angle θ_0 may be intercepted by the aperture of the lens. In Fig. 8 W_1, W_2 represent the windows enclosing the turbid liquid, and R_1, R_2 are rays defining the width of the light beam. Only particles lying within the cone *CDE* can contribute the full amount of forward scattered light, and particles at other points in the liquid have part of the scattered light intercepted by the aperture of the lens L_2. The amount of scattered light collected is obviously (*a*) less than that due to the whole volume of turbid liquid, and (*b*) greater than that due to the particles in the cone *CDE*. It is therefore possible in a simple way to set limits to the amount of scattered light collected; an exact solution would involve computing the proportion of scattered light collected from each particle outside the cone.

Let μ_t be the true attenuation coefficient of the turbid medium (as measured with a perfect instrument);

μ_a be the attenuation coefficient as measured if all the scattered light were received by the photocell (case *a*);

μ_b be the attenuation coefficient as measured if only scattered light from the cone *CDE* were received by the photocell (case *b*);

μ be the measured attenuation coefficient with the actual instrument.

Then the value of μ corresponding to a certain value of μ_t will lie between the limits μ_a and μ_b.

Calculation of μ_a and μ_b corresponding to a given value of μ_t

μ_a is simply related to μ_t. Thus:

$$\mu_a = F\mu_t. \tag{A 2}$$

To find μ_b consider a typical ray such as R in Fig. 8 which is for part of its path outside the cone *CDE* and for part inside *CDE*. The coefficient μ_b is deduced by assuming the attenuation coefficient to be μ_t for the first part of the path and $F\mu_t$ for the latter part.

It is then fairly simple to show that

$$\exp(-X\mu_b) = (1 - r_1^2/R_0^2)\exp(-X\mu_t) + \frac{2\exp[\mu_t\{-X + (1-F)r_1\cot\theta_0\}]}{R_0^2\mu_t^2(1-F)^2\cot^2\theta_0} \times \{1 - [1 + \mu_t(1-F)r_1\cot\theta_0]\exp[-\mu_t(1-F)r_1\cot\theta_0]\}, \tag{A 3}$$

where X is the path length of the beam, R_0 is the radius of the beam, and $\theta_0 = s/f$.

Application to the A.R.L. Hydrophotometer

The important dimensions are as follows:

Path length of beam in liquid	$X = 50$ cm
Radius of beam	$R_0 = 2·25$ cm
Radius of aperture in stop S_2	$s = 0·3$ cm
Focal length of lens L_2	$f = 5·3$ cm
	$\theta_0 = 3·2°$

Assume a mean wave-length = 5500Å.

Table II shows the values of μ_a and μ_b calculated from equations (A 2) and (A 3) respectively, corresponding to $\mu_t = 3{\cdot}0\ \mathrm{m}^{-1}$ for opaque particles of different diameters. For a constant size of particle μ_a is proportional to μ_t, equation (A 2), and it has been found by calculation that μ_b is also proportional to μ_t with considerable accuracy.

TABLE II

Percentage transmission per half metre 22·3 %

$\mu_t = 3{\cdot}0\ \mathrm{m}^{-1}$

Particle diameter (microns)	F	μ_a	μ_b	$\frac{1}{2}(\mu_a+\mu_b)$	k
0	1·000	3·00	3·00	3·00	1·0
2·5	0·925	2·78	2·95	2·86	1·05
10	0·585	1·76	2·72	2·24	1·34
∞	0·500	1·50	2·66	2·08	1·44

If $\frac{1}{2}(\mu_a+\mu_b)$ be taken as an approximate measure of μ a factor can be derived which will give some measure of correction for the inclusion of scattered light. This correction factor k is given by $2\mu_t/(\mu_a+\mu_b)$ approximately, and its value for each of the particle sizes computed is shown in Table II. If k be taken as 1·3 the error in k is not likely to exceed ±20 % over the size range 5 microns and upwards. For particle sizes much smaller than 5 microns the Fraunhofer diffraction theory does not apply and the electromagnetic theory of scattering must be used to determine the value of the correction factor.

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NOTE ON *CAPULUS UNGARICUS* (L.)

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(Text-figs. 1–3)

The marine gastropod *Capulus ungaricus* (L.) is found on rocks and shells, and is known often to associate with filter-feeding lamellibranchs, particularly frequenting beds of *Pecten maximus* (L.), *Chlamys opercularis* (L.) and *Modiolus modiolus* (L.). It has also been found on the shells of living *Turritella communis* Risso (Jones, 1950, unpublished) and on the superior part of the calcareous tubes of *Protula intestinum* Lam. (Lo Bianco, 1888).

Capulus itself is well known to be a ciliary feeder, and its feeding mechanism has been described by Orton (1912) and Yonge (1938). Observation of the site of several specimens on the shells of living lamellibranchs, together with the fact that the gastropod possesses a long mobile proboscis capable of great extension, led Orton (1949, 1950) to suggest that *Capulus* might also be 'semi-parasitic' on lamellibranchs, from which it could collect food by inserting the proboscis inside the bivalve shell and reaching the food in the food-grooves on the gill lamellae or on the recurrent ciliated path along the edge of the mantle. Living material was therefore collected and observed in the hope of obtaining further evidence as to the truth of this suggestion.

Twenty-nine attached specimens were obtained from off the south coast of the Isle of Man, mostly by dredging from the local beds of scallops, queens and horse mussels. Twenty-two were on the shells of living lamellibranchs, one was close to the aperture of a tube of *Pomatoceros* on the shell of a living *Chlamys opercularis*, two were on empty shells, the valves of which were still attached to each other, and four on the outer surface of single valves.

The position on the valve was recorded for twenty-six individuals, of which twenty-three were situated as previously described by Orton (1949). In its characteristic position the gastropod sits at the edge of the valve with the front margin of the shell projecting a little over it and the apex pointing inwards (Fig. 2A, C). Fig. 1 shows the sites of *Capulus* found on *Chlamys opercularis*. More *Capulus* were on the right valve, which is the flatter of the two and lies undermost when *Chlamys* is living, than on the left. The majority had attached themselves to the anterior half of the margin of the valve, only three out of fifteen being on the posterior side; the smallest of these was that situated by the aperture of a *Pomatoceros* tube, partly on the *Chlamys* and partly on the tube itself, and the other two were on single valves. None have yet been found on the posterior auricle. The numbers are few, but suggest a possible preference for the anterior edge from the auricle and byssal notch round the ventral curve of the margin. Thus almost all the *Capulus* were on that part of the circumference of the valve where the wide inhalant current of *Chlamys* would be operative and away from the powerful exhalant current which goes out posteriorly over a more restricted region. *Capulus* on *Pecten maximus* (Fig. 2C) and *Monia patelliformis* (L.) were similarly placed. On *Modiolus modiolus*, *Capulus* were on the ventral edge, above or posterior to the emergence of the byssus, again away from the exhalant current (Fig. 2A).

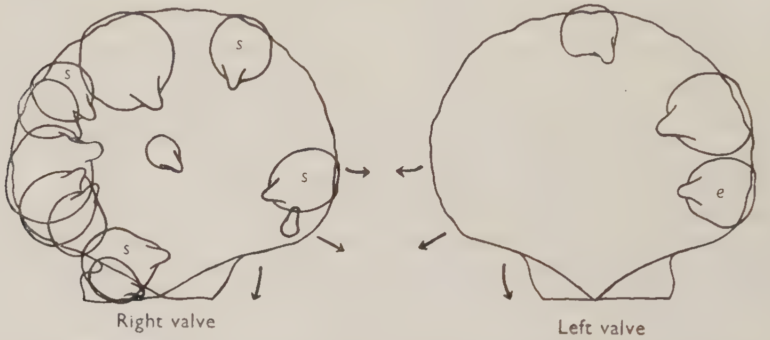

Fig. 1. Composite diagram showing positions of *Capulus ungaricus* on *Chlamys opercularis*. *e*, on empty shell, the valves of which were still attached to each other; *s*, on single valves. Otherwise on shells of living *Chlamys*. Arrows show direction of outgoing currents of *Chlamys*.

When *Capulus* is removed from its attachment, the site often displays a scar which may vary from just a cleaner patch, the sculpture of the valve being unaffected, to a well-defined, almost circular mark with a grooved margin within which the sculpture of the valve is destroyed (Fig. 2B). The circumference of the scar corresponds to that of the aperture of the animal's shell, and the grooved margin sometimes found suggests that the edge of the calcareous part of the shell works on the valve and mechanically produces the groove. The shell of *Capulus* has a fringed periostracum prolonged well beyond the calcareous edge, and this border fits closely on to the undulations of the valve beneath, except at the anterior margin, where the shell projects a little beyond the edge of the valve. In this region there is often a semi-lunar gap in the valve edge, as was first noticed by Orton (1949) in the case of a single specimen on *Monia*. It is sometimes difficult to assess the genuineness of this gap, as *Capulus* on a ribbed shell may be so placed that the mid-anterior margin is opposite a ridge on the outer surface, i.e. opposite a natural inward undulation of the valve, and valves are often chipped. But in some cases the outer layers of the shell are recessed farther back than the inner, which suggests that the *Capulus* itself was responsible for the gap rather than that it settled, by preference or chance, over such a gap already there. In this connexion I have once seen a *Capulus* remove a piece of shell from the edge of a *Pecten* valve by means of its radula.

The majority of those *Capulus* which had been settled sufficiently long to leave a scar had shown no change in position when kept in sea water in aquarium tanks for periods of up to 3 months, but some power of movement is retained; an animal 39 mm in length, the largest found, was able when freed from its attachment to climb up the side of its container for a short distance, and while some of the smaller ones revealed evidence of long settlement others frequently moved off their attachments, leaving no scar, or were brought up free in the dredge.

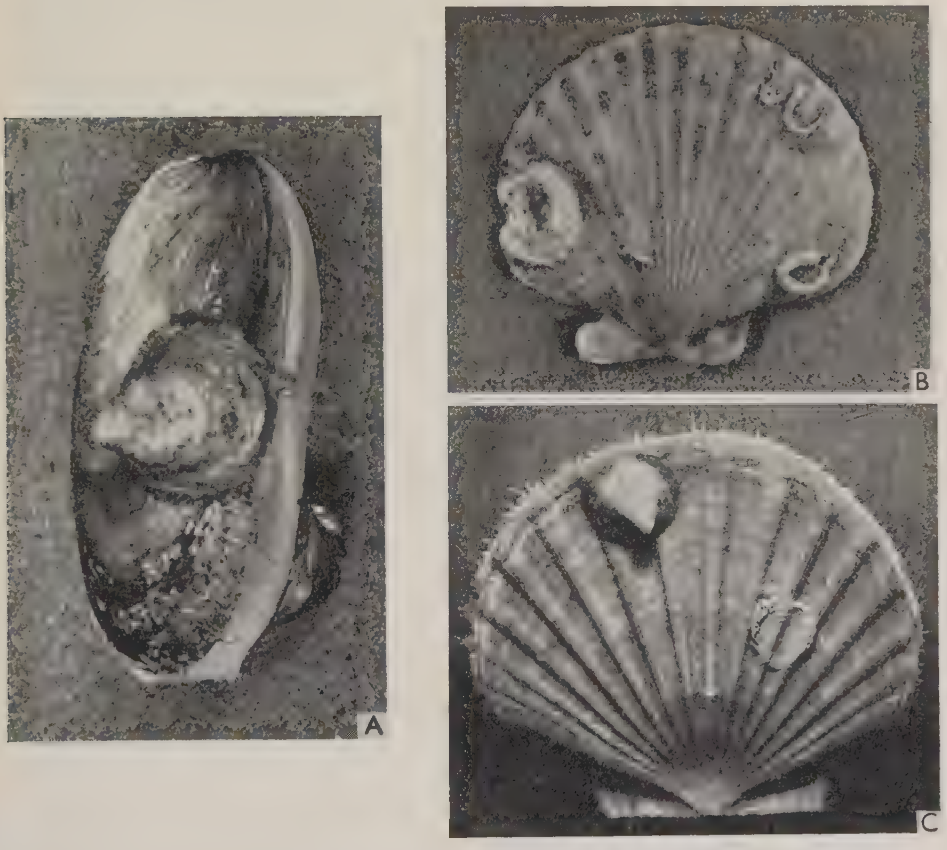

Fig. 2. A: two *Capulus ungaricus* on the shell of a living *Modiolus modiolus*, one on each valve: length of *Modiolus* shell, 62 mm. B: scar left by *Capulus ungaricus* on shell of *Chlamys opercularis*: the shell of the *Chlamys* measures 77 mm from apex to mid-ventral margin. C: *Capulus ungaricus* on the shell of a living *Pecten maximus*: the shell of the *Pecten* measures 120 mm from apex to mid-ventral margin.

From this it would appear that while after metamorphosis there is a mobile phase during which the animals crawl actively on the substrate, they tend to settle and remain in later life on the shells of ciliary feeders in a position which would give them the opportunity of using the proboscis to collect food as previously suggested.

The so-called proboscis is in fact a prolongation of the lower lips of the animal to form an almost cylindrical structure having a dorsal groove which runs longitudinally from the soft tip to the mouth with the radular apparatus at the base. The proboscis is not eversible, and is often held curled round to left or right and hidden between head and propodium, from the dorsal surface of which it is stated to take up food particles in mucus, brought thither by the animal's own ciliary feeding currents (Yonge, 1938). According to Yonge, the radula appears to have essentially a conveying function. Peile's observation (1937) that the teeth are slightly blunted in a few of the front rows may possibly be correlated with the formation of the gap mentioned above. On one occasion, when suspended particles of carmine were being used to demonstrate the ciliary currents of a *Capulus* attached to *Chlamys*, the lips diverged and the radula was seen, appearing to grasp a string of particles as does that of *Crepidula fornicata* (L.), but also the string of particles appeared at one time to go smoothly into the mouth, without the emergence of the radula. Fig. 3 shows a sketch made on this occasion; one valve of the *Chlamys* had been removed, and the corresponding lobe of the mantle reflected. The head and cephalic tentacles of the *Capulus* can be seen in the gap between the two shells, over which gap the mantle tentacles of the *Chlamys* partially extend. The

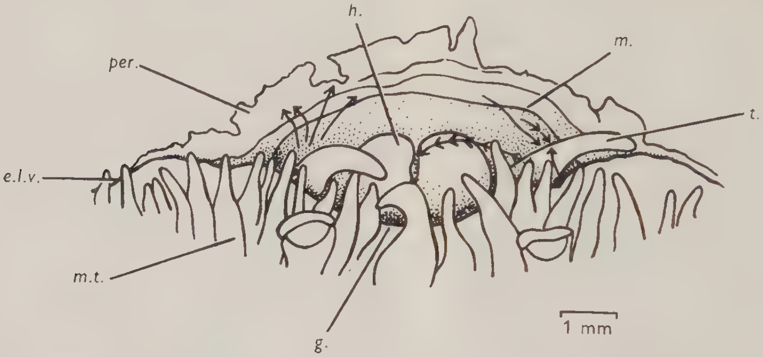

Fig. 3. *Capulus ungaricus* attached to shell of living *Chlamys opercularis*. Right valve of *Chlamys* removed and corresponding lobe of the mantle and gill reflected. Arrows indicate the directions of the ingoing and outgoing currents of *Capulus*, and continuous arrows a string of particles passing over the left head lobe to the mouth. *e.l.v.*, edge of left valve of shell of *Chlamys*; *g.*, gap in the margin of the *Chlamys* valve; *h.*, head of *Capulus*; *m.*, mantle of *Capulus*; *m.t.*, mantle tentacles of *Chlamys*; *per.*, periostracum of *Capulus*; *t.*, cephalic tentacle of *Capulus*.

arrows indicate the directions of the inhalant and exhalant currents of the *Capulus*, as far as they could be seen in this view, and the string of particles passing into the mouth. The current of the *Capulus* also drew down particles from the region of the mantle tentacles of the *Chlamys*.

From such a position the proboscis could be inserted between the valves of a feeding lamellibranch, and in fact has now been observed to be so inserted in the case of a *Capulus* on the shell of a living *Chlamys opercularis*. The proboscis was seen on four occasions to be extended through the gap, over the edge of the *Chlamys* valve, and in between the margins of its velum. It therefore seems clear that *Capulus* may feed on those planktonic constituents already collected by the *Chlamys* on which it sits. The relationship appears, however, to be facultative. Not only have *Capulus* lived for many weeks on single valves in the aquaria at Port Erin and Cullercoats, where their only source of food would be those particles collected by their own ciliary feeding mechanism, but also they have been regularly brought up on rocks and stones in regions off the Northumberland coast where their usual bivalve 'hosts' are scarce (Dr H. O. Bull, personal communication).

As regards the effect on the bivalve, the probable removal of some of its food supply has no obvious detrimental effect, for *Pecten*, *Modiolus* and *Chlamys* bearing *Capulus* have lived for months in aquarium tanks. The velum and velar tentacles of *Chlamys* did not appear to be affected in any way by the insertion of the proboscis, which did not even cause them to retract.

This work was carried out while holding a Herdman Studentship of the University of Liverpool.

SUMMARY

Capulus ungaricus on the shells of living lamellibranchs occupies a characteristic position, at the edge of the valves, away from the exhalant current, and with the anterior margin overlying the valve edge. The valve is scarred by the continued presence of the gastropod and the site of the latter is often associated with a semi-lunar gap in the valve edge.

Insertion of the proboscis of *Capulus* through this gap and between the vela of *Chlamys opercularis* has been observed, thus strongly supporting Orton's suggestion that the gastropod might utilize its position to become semi-parasitic. The association appears to be facultative and to have no obvious detrimental effect upon the 'host'.

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STRONTIUM AND BARIUM IN SEA WATER AND MARINE ORGANISMS

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(Text-fig. 1)

Since the advent of nuclear fission, considerable interest has been aroused in the biological fate of strontium, as two isotopes of this element are among the longer-lived fission products of uranium. Strontium 90, in particular, constitutes the major hazard in old fission products because it is readily taken up by all kinds of plants and may then be concentrated in the bones of animals feeding on the plants. Strontium 90 contamination may be derived from fall-out from atomic explosions or from uncontrolled disposal of fission products. The oceans should receive more than twice as much Strontium 90 from fall-out as the continents because 70% of the surface area of the globe is covered by sea. The oceans are also used, though to a very limited extent at present, for the disposal of fission products from reactor wastes. Hence it is obviously important to study the behaviour of strontium in marine organisms in order to evaluate the health hazard caused by controlled or uncontrolled radioactive contamination of sea water. Barium has only relatively short-lived fission-product isotopes, but can readily be studied at the same time as strontium because of its similar chemistry.

Strontium and barium are elements which have long been known to occur in marine organisms and the sea. In the past, however, there have been considerable difficulties in the analytical determination of these two elements. Strontium, especially in trace amounts, is very difficult to separate from calcium, and while barium is more easily separated it is present in amounts so small as to tax the ingenuity of the conventional analyst. Spectrometric analysis has been used by several workers to determine these elements, but is not always sufficiently sensitive nor very precise. Recently, Harrison & Raymond (1955) and Bowen & Dymond (1955) have described the method of activation-analysis for these elements in animal and vegetable tissues respectively. In this work the same technique has been applied to determine strontium and barium in sea water and in various marine organisms.

METHODS

Collection and preparation of specimens

Sea water was collected and stored in polythene aspirators and all chemical manipulations were carried out using polythene apparatus. The chlorinity was determined by the Mohr titration method. Strontium was determined by activating 2 ml. samples of sea water sealed in polythene tubes with neutrons in the Harwell pile, BEPO. The associated activity, largely ^{24}Na and ^{38}Cl, after activation was considerable, and repeated precipitation with sodium carbonate was used to ensure complete decontamination from active sodium and chlorine. ^{139}Ba was separated from the ^{87}Sr, but could not be counted from such a small volume of sea water. Hence barium was concentrated from 1·5 l. samples of sea water using purified Zeo-Karb 225, sulphonated polystyrene resin. Columns of this resin, 15 cm long and 1 cm diameter, were set up in polythene tubes and were washed successively with 500 ml. 2N-NH_4Cl at pH 3·0, 500 ml. 6N redistilled HCl and finally 200 ml. redistilled H_2O. The NH_4Cl was made by passing cylinder ammonia into redistilled HCl in a polythene bottle. After this treatment samples of the resin left no residue after ashing at 500° C. Sea water 'spiked' with ^{140}Ba was then used to determine the break-through-volume of barium for the columns used. It was established that 88 % of the barium in 1·5 l. of sea water was retained by the resin-column using a flow-rate of 2 ml./min. The losses were made up as follows:

Passed through resin	Percentage Ba lost
500 ml. 'spiked' sea water	0·4
1000 ml. 'spiked' sea water	1·9
1500 ml. 'spiked' sea water	8·3
500 ml. N/5 HNO_3 wash	3·8

After passing 1·5 l. of sea water through the column the resin was washed with 500 ml. of 0·2N redistilled HNO_3 and allowed to drain. Practically all the sodium, potassium and chlorine were removed in the eluent. The resin was then transferred to a silica basin and ashed at 500° C for 24 hr. The barium in the ash was determined by neutron-activation, taking care to remove sulphate ions from the activated ash by repeatedly boiling with saturated sodium carbonate solution and extracting the residue with hydrochloric acid. A 'blank' analysis of the clean resin showed no detectable barium.

Marine algae were collected in polythene bags and analysed as soon after collection as possible. Samples of tissue free from encrustations and parasitic algae were selected and washed with dilute choline chloride solution. They were then blotted dry and ashed in silica basins at 500° C: samples of the ash were used for activation-analysis.

Corals and mollusc shells were washed with distilled water, dried and ground in an agate mortar. They were not ashed before analysis.

Analytical methods

The method of activation-analysis has already been described (Harrison & Raymond, 1955; Bowen & Dymond, 1955). In this work ^{87}Sr was counted in liquid form in a scintillation counter as previously described, but ^{139}Ba was generally slurried on to a counting tray as the solid chromate, dried, and counted with an end-window EHM2 Geiger-Muller counter. Chemical yields were better than 95% for strontium and 90% for barium. Determinations of calcium were made by flame-photometry as described by Bowen & Dymond (1955).

RESULTS

Table I gives the strontium and barium contents of sea water from the English Channel corrected to 19·0‰ chlorinity.

TABLE I. STRONTIUM AND BARIUM CONTENT OF SEA WATER

Vol. sea water taken	mg Sr/l.	Vol. sea water taken	μg Ba/l.
400 ml. abs. on resin	8·66	900 ml. abs. on resin	5·8
2 ml. abs. on resin	8·69	1500 ml. abs. on resin	5·9
2 ml. abs. on resin	8·30	1500 ml. abs. on resin	6·8
2 ml. abs. on resin	8·36		
Mean	$8{\cdot}50_2$	Mean	6·2

These figures should require a small correction because of the presence of uranium in sea water: ^{235}U undergoes nuclear fission on neutron activation and ^{92}Sr and ^{139}Ba are produced. The concentration of uranium in sea water is

TABLE II. ALKALINE EARTH CONTENT OF MARINE ALGAE

		p.p.m. dry wt.			wt./wt.	
Species	Where collected	Ca	Sr	Ba	Ca/Sr	Ca/Ba
Brown						
Fucus serratus L.	Plymouth	8,430	804	14	10·5	800
F. serratus L.	Weymouth	8,270	833	<23	9·9	>360
F. vesiculosus L.	Weymouth	8,180	702	9·5	11·6	860
Laminaria digitata Lam.	Plymouth	9,520	1,045	6·5	9·1	1,500
L. digitata Lam.	Weymouth	7,080	783	<23	9·0	>310
L. saccharina Lam.	Weymouth	7,720	698	11·3	11·1	680
Ascophyllum nodosum Le Jol.	Weymouth	8,320	428	5·6	19·5	1,500
Chorda filum Lam.	Weymouth	16,300	1240	18·6	13·2	880
Mean		9,228	817	11	12	1,040
Red						
Gigartina stellata Batt.	Plymouth	2,810	133	2·1	21	1,300
Chondrus crispus Lyngb.	Plymouth	6,420	131	5·6	49	1,100
Rhodymenia palmata Grev.	Weymouth	1,050	18·8	0·6	56	1,700
Mean		3,427	94	2·8	42	1,370
Green						
Enteromorpha compressa Grev.	Plymouth	10,300	87	—	120	—
E. intestinalis Link.	Weymouth	4,590	54·8	0·4	84	11,000
Ulva lactuca L.	Weymouth	3,840	38·2	2·5	100	1,500
U. lactuca L.	Plymouth	4,560	67·7	—	68	—
Mean		5,822	62	1·45	93	6,250

about 1·5 μg/l. (Föyn, Karlik, Pettersson & Rona, 1939), and this would give rise to an apparent 0·0008 mg/l. of strontium and 0·2 μg/l. of barium.

Hence the correction is negligible for strontium and within the accuracy of the analysis for barium.

Tables II–IV give calcium, strontium and barium analyses for sea weeds, molluscan shells, and corals respectively.

Table III. Alkaline Earth Content of Mollusc Shells

Species	Where collected	p.p.m. dry wt.			wt./wt.	
		Ca	Sr	Ba	Ca/Sr	Ca/Ba
Amphineura						
Acanthochitona crinitus Penn.	Weymouth	239,000	3,950	< 10	60	> 24,000
Lepidochitona cinereus L.	Weymouth	317,000	5,600	20	57	16,000
Mean		278,000	4,775	—	58·5	—
Gastropoda						
Patella vulgata L.	Weymouth	402,000	2,320	< 4	173	> 100,000
Littorina littoralis L.	Weymouth	388,000	1,720	< 5	225	> 80,000
Haliotis tuberculata L.	Sark	412,000	2,340	< 7	176	> 60,000
Rìssoa parva Da Costa	Lands End	313,000	1,520	< 6	206	> 50,000
Bittium reticulatum Da Costa	Kimmeridge	361,000	1,420	< 4	248	> 90,000
Scaphopoda						
Dentalium vulgare Da Costa	Herm	406,000	3,550	7·2	114	60,000
Lamellibranchiata						
Arca tetragona L.	Lands End	353,000	3,260	< 4	108	> 90,000
Cardium echinatum L.	Weymouth	386,000	2,260	< 1	170	> 400,000
Pecten maximus L.	Weymouth	404,000	1,630	< 3	247	> 130,000
Lasaea rubra Mont.	Kimmeridge	375,000	1,820	< 25	206	> 15,000
Teredo megotara F. & H.	Weymouth	355,000	1,810	48	196	7,000
Mean		377,700	2,150	—	188	—
Cephalopoda						
Sepia elegans D'Orbigny	Lands End	243,000	2,910	20	84	12,000

Table IV. Alkaline Earth Content of Recent Corals

Species	Where collected	p.p.m. dry weight			wt./wt.	
		Ca	Sr	Ba	Ca/Sr	Ca/Ba
Millepora sp.	Indian Ocean, 8 fm.	323,000	7,360	8	44	40,000
Allopora norvegica	N. Atlantic, 87 fm.	303,000	7,760	18	39	17,000
Heliopora coerulea	China Sea, 13 fm.	325,000	6,520	85	50	4,000
Caulastraea furcata	China Sea, 32 fm.	324,000	13,400	11	24	30,000
Hydnophora polygonata	China Sea, 6 fm.	310,000	10,700	24	29	13,000
Pocillophora verrucosa	China Sea, 13 fm.	327,000	12,900	11	25	30,000
Psammocora planipora	Indian Ocean, 60 fm.	295,000	5,590	23	53	13,000
Seriatopora elegans	China Sea, ½ fm.	332,000	12,500	11	27	30,000
Leptoseria solida	Indian Ocean, 60 fm.	326,000	8,680	12	38	27,000
Acropora sp.	Persian Gulf, 63 fm.	306,000	8,970	56	34	5,500
Alveopora sp.	Indian Ocean, 39 fm.	296,000	7,840	210*	38	1,400
Merulina sp.	China Sea, 30 fm.	337,000	8,360	450*	41	750
Stylopora sp.	Muscat, 1 fm.	323,000	8,490	43	37	7,500
Montipora sp.		345,000	9,290	—	37	—
Amphihelia sp.		345,000	10,650	—	32	—
Mean		321,100	9,267	29·5	36	16,860

N.B. The two starred barium figures were omitted in taking the mean.

Fossil corals were also investigated and the results are collected in Table V.

TABLE V. ALKALINE EARTH CONTENT OF FOSSIL CORALS

			p.p.m. dry wt.			wt./wt.	
Species	Where collected	Geological age	Ca	Sr	Ba	Ca/Sr	Ca/Ba
Dendrophyllia elegans		Oligocene	361,000	8300	66	44	5,500
Balanophyllia calyculata		Upper chalk	326,000	6850	251	47	1,300
Caryophyllia cylindracea	Wilts	Upper chalk	335,000	640	<5	523	>67,000
Caryophyllia sp.		Upper chalk	345,000	526	35	658	10,000
Coelosmilia regularis	Wilts	Upper chalk	310,000	524	<9	591	>34,000
Holocystis elegans	Isle of Wight	Lower greensand	83,900	278	<21	301	>4,000
Micrabacia coronula	Yorks	Upper chalk	270,000	273	8	990	34,000
M. fittoni	S. England	Gault	265,000	4230	160	63	1,700
Parastraea stricta		Cretaceous	309,000	7640	18	41	17,000
Placosmilia rudis	Lérida, Spain	Cretaceous	312,000	1760	<15	177	>21,000
Trichosmilia granulata		Cretaceous	369,000	530	15	695	25,000
Chromatoseris porpites	Boulogne, France	Jurassic	309,000	209	<19	1040	>16,000
Dimorphoseris terquenni	Glos	Jurassic	384,000	295	<6	1300	>64,000
Goniocera socialis	Wilts	Jurassic	335,000	1860	<220	180	>1,500
Isastraea explanata	Berks	Jurassic	391,000	1500	<19	260	>21,000
I. explanata	Berks	Jurassic	365,000	1130	—	322	—
I. limitata	Dorset	Jurassic	337,000	730	<10	461	>34,000
Montlivaltia delabecki	Glos	Jurassic	313,000	538	<28	583	>11,000
M. dispar	Berks	Jurassic	259,000	1356	—	184	—
Oppelismilia mucronata	Leics	Lower Lias	269,000	972	20	276	13,000
O. victoriae	Oxon	Middle Lias	254,000	548	<66	465	>4,000
Rhabdophyllia phillipsii	Berks	Jurassic	386,000	219	<4	1760	>100,000
Stylina tubulifera	Wilts	Jurassic	375,000	1540	<210	243	>1,800
Thamnasteria arachnoides	Wilts	Jurassic	351,000	437	—	802	—
T. concinna	Berks	Jurassic	398,000	1180	<2	338	>200,000
T. defranciana	Glos	Jurassic	330,000	575	<3	574	>110,000
Thamnasteria sp.	Berks	Jurassic	353,000	810	—	435	—
Theocosmilia annularis	Wilts	Jurassic	371,000	678	<11	549	>34,000
Dibunophyllum bipartitum	Glos	Carboniferous	277,000	418	8	661	35,000
Lithostrotion decipiens	Glos	Carboniferous	196,000	155	<31	1270	>6,000
L. martini	Pembs	Carboniferous	367,000	154	11	2380	33,000
L. pauciradiale	Somerset	Carboniferous	362,000	383	<22	944	>16,000
L. portlocki	Westmorland	Carboniferous	365,000	398	4	917	91,000
Lithostrotion sp.		Carboniferous	353,000	304	—	1160	—
Lonsdaleia floriformis	Glos	Carboniferous	358,000	537	<27	658	>13,000
Michelinia favosa	Pembs	Carboniferous	241,000	<850	17	233	14,000
M. megastoma	Westmorland	Carboniferous	305,000	902	5	338	61,000
Palaeosmilia murchisoni	Glos	Carboniferous	375,000	308	<6	1250	>62,000
P. regia	Glos	Carboniferous	356,000	733	<44	486	>8,000
Alveolites billingsi	W. Ontario	Devonian	—	364	6	—	—
A. cervicornis	Devon	Devonian	389,000	157	7	2480	56,000
Cystiphyllum vesiculosum	Devon	Devonian	113,000	489	35	230	3,200
Favosites goldfussi	Devon	Devonian	400,000	675	3	592	130,000
F. stricklandi	Salop	Silurian	354,000	842	—	418	—
Halysites catenularius	Worcs	Silurian	305,000	1780	<26	172	>12,000
H. catenularius	Salop	Silurian	327,000	973	10	336	33,000
Kodonophyllum truncatum	Salop	Silurian	340,000	828	7	411	50,000

DISCUSSION

Our value for the amount of strontium in sea water is 5% higher than recent flame spectrometric determinations by Odum (1951*a*) and Chow & Thompson (1955), and an activation determination by Hummel & Smales (1956). The discrepancy may arise since our sample was collected near the shoreline whereas the other workers used oceanic water, but it is within the limits of accuracy of both methods. Our value for barium is considerably lower than has been previously reported. Thus Thompson & Robinson (1932) report 200 μg/l., and von Engelhardt (1936) found 54 μg/l. by a spectrometric method. The discrepancy is not surprising in view of

Goldschmidt's remarks (1937); 'In several cases we have found that in traditional methods of analytical determination of minor constituents a higher percentage of, for instance, barium, has been contributed from the reagents than from the specimen'. The mean of the published results for calcium in sea water of 19‰ chlorinity is 403 mg/l. (Harvey, 1955), so we find the Ca/Sr and Ca/Ba ratios in sea water to be 47·4 and 65,000 (wt/wt) respectively.

Relatively little work has been reported on the alkaline earth contents of marine organisms. Forchammer was the first worker to detect strontium (1859) and barium (1884) in the ash of *Fucus vesiculosus*, a species which later work has shown to be an accumulator and a discriminator for both elements. All the later analytical work has been spectrometric in nature. Fox & Ramage (1931) found strontium in nearly all the soft tissues of marine invertebrates they examined, but could not detect barium. Webb (1937) showed that two brown algae, *Fucus serratus* and *Saccorhiza bulbosa*, both discriminate for strontium and barium, while most of the seven molluscs studied discriminated against strontium. The mantle of *Archidoris britannica* is remarkably rich in strontium, which is interesting in view of the recent observations of Fretter (1953) and Rao & Goldberg (1954) that alkaline earths are absorbed largely through the mucus of marine invertebrates. McCance & Masters (1937) also record concentration of strontium by *Archidoris*. Analyses of algae by Borovik-Romanova (1939), Wilson & Fieldes (1941), and Black & Mitchell (1952) are of the same order as those found here. The last authors are responsible for the interesting observation that the strontium-content (p.p.m. dry matter) of all the algae studied diminished by a factor of 3–6 between January and June.

Shells of marine molluscs have been analysed for strontium by Vinogradov (1937) and for barium by Borovik-Romanova (1939) (see also Vinogradov, 1953). Earlier, Thomas, quoted in Thompson & Robinson (1932), stated that the Ca/Sr ratio in shells is roughly equal to the ratio in sea water. Our results do not support this contention, nor do those of Odum (1951*b*) who found a mean Ca/Sr ratio of 174 in a number of recent mollusc shells, in good agreement with our figures. Odum also showed that the shells of brachiopods discriminate against strontium about as much as molluscs: recent corals were found to have a mean Ca/Sr of 43, again in agreement with the present work.

The terms 'accumulation' and 'discrimination' as applied to the uptake of nutrient elements need clear definition, since different authors use these words in different senses. Black & Mitchell (1952) define an 'accumulation factor' which we will call A here, as

$$A=\frac{\text{p.p.m. element in dry organism}}{\text{p.p.m. element in sea water}},$$

while Spooner (1949) uses a more complex definition based on the effective dilution of sea water in the element per g of organism. In this work we shall define a 'discrimination factor D' for the uptake of a pair of related elements X and Y as:

$$D=\frac{X/Y \text{ in dry organism}}{X/Y \text{ in sea water}},$$

so that clearly

$$D(X,\ Y)=A(X)/A(Y).$$

The rather unwieldy data in Tables II–IV can be summarized in terms of mean accumulation and discrimination factors for the different groups (Table VI). Hence we see that all the organisms studied concentrate calcium, strontium and barium. Marked discrimination for strontium and barium occurs in the brown algae; the other algae discriminate for barium but not strontium. Molluscan shells, unlike corals, discriminate against both strontium and barium.

TABLE VI. MEAN ACCUMULATION AND DISCRIMINATION FACTORS FOR MARINE ORGANISMS

Group	No. studied	A (Ca)	A (Sr)	A (Ba)	D (Sr, Ca)	D (Ba, Ca)
Brown algae	8	23	96	1800	4·2	78
Red algae	3	8·5	11	450	1·3	53
Green algae	4	14·5	7·3	—	0·50	16
Chiton shells	2	690	560	2400	0·81	3·5
Cephalopod bone	1	600	340	3200	0·57	5·3
Other mollusc shells	11	940	250	650	0·27	0·7
Corals	15	800	1090	4400	1·4	5·5

The amount of ^{90}Sr which will ultimately reach the ocean from all atomic explosions to date is estimated to be about 0·8 megaCurie. If this were uniformly mixed into the ocean, which it certainly is not, this would amount to $5\times 10^{-12}\,\mu$c/ml. Since $10^{-1}\,\mu$c is about the smallest amount of β-activity that can be detected experimentally, 1000 kg of sea water would have to be concentrated down to determine this isotope. However, it should be possible to measure ^{90}Sr in kilogram samples of coral, since corals accumulate the element by a factor of 1000. This would constitute a very delicate test for the isotope in any given area of ocean. It might be possible to determine the rate of mixing of the oceans by measuring the ^{90}Sr content of corals from different depths. The hazard from contamination of the ocean by radioactive strontium is negligible at present, in view of the enormous quantities of naturally radioactive substances already existing there, e.g. nearly 500,000 megaCuries of ^{40}K alone.

The mechanism of accumulation of ions is fairly obvious. Large volumes of sea water are strained of their ions by natural ion-exchange systems in animals and sea weeds, and these ions may then be precipitated as insoluble inorganic structures (as is calcium in molluscs and corals) or retained in the ion-exchange systems (as in sea weeds). In this connexion it is interesting to notice that in order to increase in weight by 1 g., a coral needs to filter all the calcium ions from 800 ml. of sea water. The barium content of the coral shows that it must have taken up all the barium from 4400 ml. of water, so that the efficiency of calcium uptake must be less than or equal to 800/4400 or 18 %. The accumulation factors of calcium and barium for molluscan shells are nearly equal, so that for these organisms the efficiency of calcium uptake may be as high as 100 %. It is much more difficult to account for the discrimination between similar ions which we have found here. It is known, however, that ion-exchange resins always discriminate for the rarer of a pair of similar ions, and it is noteworthy that the very scarce ion, barium, is retained more strongly than the more common ion strontium in almost every case.

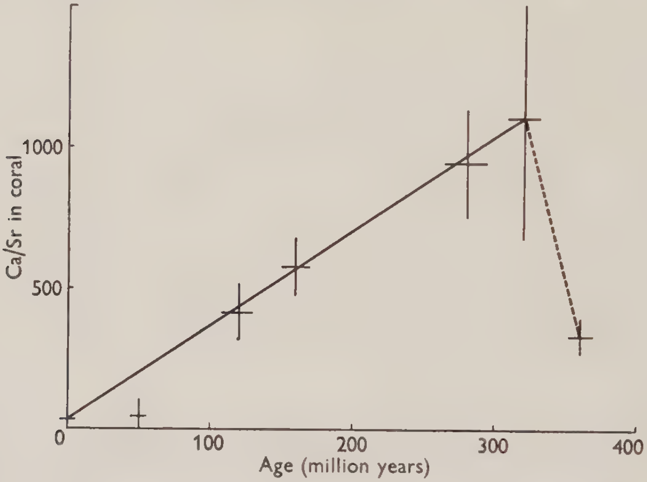

Fig. 1. Apparent Ca/Sr in corals plotted against geological age.

During inorganic precipitations traces of foreign ions are generally left in the mother liquor so that the precipitate is purer than the original solution. Barium and strontium are likewise excluded in the biochemical precipitation of calcium carbonate by molluscs, but not by corals. Some of the molluscs studied were constructed of calcite and others of aragonite, but we could find no consistent difference in strontium and barium content between the two forms. Theoretically one would expect strontium to prefer the aragonite structure to calcite.

The fossil corals were studied in order to check the results of Odum (1951*b*) and Kulp, Turekian & Boyd (1952) regarding the constant level of strontium in the ocean during geological time. These authors analysed a number of recent and fossil molluscs and brachiopods by a spectrometric method and reported that the Ca/Sr ratio remained constant at roughly 175/1 in all the specimens. We have two comments to make on this work.

In the first place molluscs and brachiopods are perhaps not the most suitable organisms for this kind of work since they discriminate against strontium by a factor of four. We have chosen corals since they appear scarcely to distinguish strontium from calcium. Secondly, the present day Ca/Sr ratio in sea water (50/1) is much smaller than in any common igneous or sedimentary rock, where it ranges from 120/1 to 1000/1. This suggests that strontium, like sodium and magnesium, is an element which has accumulated in the sea during the earth's history.

Our results are summarized in Fig. 1. They indicate a progressive increase in the Ca/Sr ratio with geological age, passing backwards in time as far as the Devonian period. The Devonian and Silurian results are of limited value because few samples of this age could be obtained. In addition, it is obvious that in the older samples the Ca/Sr ratios may have been drastically altered from their original values by recrystallization. This may well apply to the four Silurian corals analysed, as there is no reason to suppose a catastrophic change in the Ca/Sr ratio in the ocean between Devonian and Silurian times. Otherwise it appears that strontium has been steadily accumulating in the ocean during geological time.

Summary

Strontium and barium have been determined in sea water. The strontium content of sea water was found to be 8·5 mg/l., in agreement with recent work, but the barium content (6·2 μg/l.) is lower than previously reported values.

Strontium and barium have also been determined in a number of marine algae, mollusc shells and corals. All the organisms studied concentrate these elements from sea water, but whereas brown algae discriminate markedly for them in preference to calcium, mollusc shells discriminate against them.

The amounts of strontium found in fossil corals are very much smaller than they are in recent corals, and there is an approximately linear relation between the Ca/Sr ratio and geological age as far back as the Devonian period: the Silurian results are anomalous. It is suggested that strontium is an element which accumulates in sea water, though further work is needed to establish the point.

I should like to thank Dr E. J. Bowen, Mr J. M. Edmonds, Dr H. D. Thomas and Mr E. White for supplying most of the specimens for analysis, and Miss J. A. Dymond for much technical assistance.

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PLANKTONIC EVIDENCE FOR IRREGULAR FLOW THROUGH THE IRISH SEA AND NORTH CHANNEL IN THE AUTUMN OF 1954

By D. I. Williamson
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(Text-fig. 1)

During the autumn of 1954 plankton samples were taken in the northern Irish Sea and the North Channel from R.V. *Clupea* (Scottish Home Department) as part of a larger survey of the distribution of herring larvae. At the same period similar samples were taken in the central Irish Sea from R.V. *William Herdman* (Marine Biological Station, Port Erin). The present paper concerns the distribution, as shown by both sets of samples, of a few selected species.

I am indebted to all those on both vessels who assisted in taking the samples and to the Marine Laboratory, Aberdeen, for lending me for analysis the samples taken from R.V. *Clupea*.

Methods

In each vessel oblique hauls were taken with a 1 m diameter silk net of 60 meshes to the inch (24 meshes per cm), weighted with a 56 lb (25 kg) iron weight. The relationship between towing depth and length of warp was determined by previous experiment with 'Sealax' sounding tubes. Each haul was taken with the vessel travelling at about 2 knots (3·5 km/h); the net was lowered rapidly to within a few fathoms of the bottom, then hauled steadily at about 9 fathoms (16 m) of warp per minute.

The dates of sampling are shown in Fig. 1 (top left). Within each sub-area shown no pair of neighbouring samples was separated by more than 24 h. Excluding the area to the south of the Isle of Man, all samples were taken within 9 days, and no pair of neighbouring samples was separated by more than 5 days. The stations to the south of the Isle of Man were added after a considerable time lag, so that the samples taken on 14 October were separated by an interval of 17 days from those immediately to the north of them, and the most southerly samples were added another 8 days later. Intervals of more than 7 days between neighbouring samples are signified by broken lines in the three distribution charts (Fig. 1).

The distributions of *Biddulphia sinensis* (Greville), *Sagitta setosa* J. Müller and *Isias clavipes* Boeck are indicated by contouring (Fig. 1). In each chart each contour level is set at 10 times that below it. With the contour levels set at such extremely wide intervals (cf. Cushing, 1953) sampling errors are likely to have little effect on the apparent distributions.

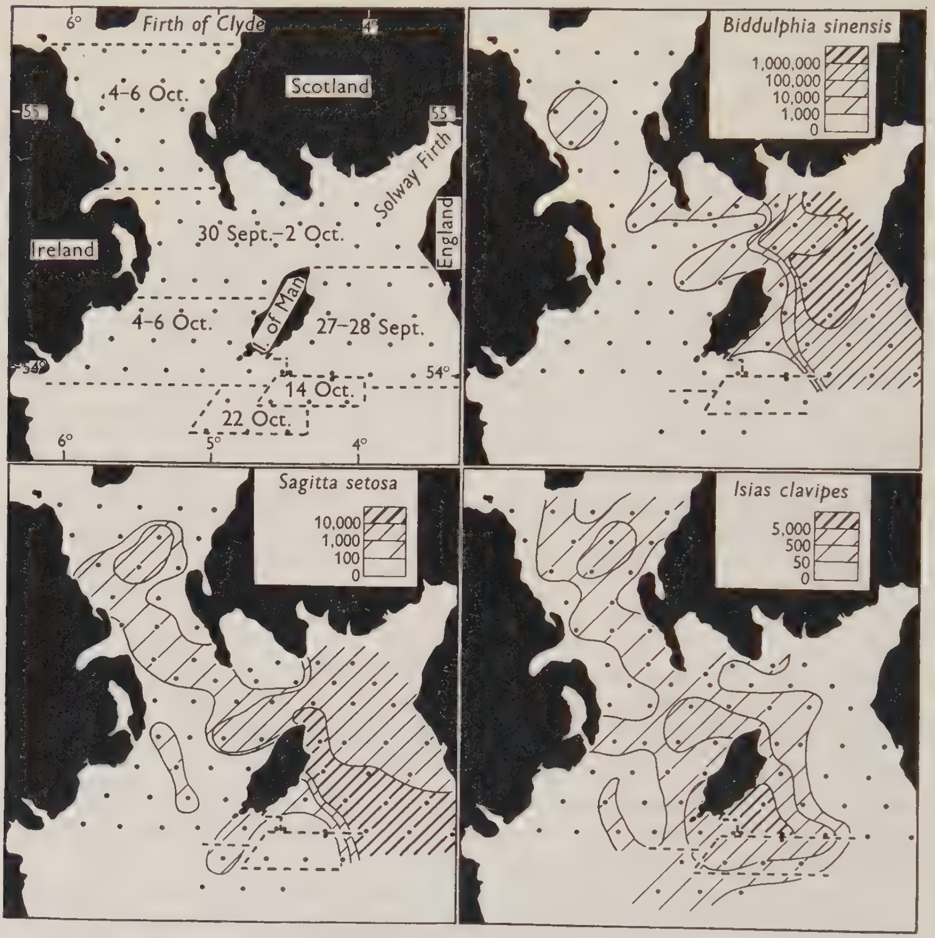
6°
Firth of Clyde
4°
Scotland
55
55
4–6 Oct.
Solway Firth
Ireland
England
30 Sept.–2 Oct.
I. of Man
4–6 Oct.
27–28 Sept.
54°
54°
14 Oct.
22 Oct.
6°
5°
4°
Biddulphia sinensis
1,000,000
100,000
10,000
1,000
0
Sagitta setosa
10,000
1,000
100
0
Isias clavipes
5,000
500
50
0

Fig. 1. Top left: North Channel and part of Irish Sea, showing positions and dates of samples. Top right: distribution of *Biddulphia sinensis*. Bottom left: distribution of *Sagitta setosa*. Bottom right: distribution of *Isias clavipes*. Keys relate to numbers of specimens per haul.

Results and Discussion

Results of the present and earlier plankton surveys (Williamson, 1952, 1956) are all consistent with the following generalized picture of conditions in the Irish Sea in the autumn. Water enters the southern Irish Sea from St George's

Channel and carries with it *Sagitta elegans*, large numbers of *Isias clavipes* and smaller numbers of other species including *Centropages typicus* and the late larvae of *Nyctiphanes couchii*. The main flow passes to the east of the Isle of Man, where it comes into contact with water very rich in *Sagitta setosa* and *Biddulphia sinensis*. There is considerable mixing in this region, and the north-flowing water itself becomes rich in *Sagitta setosa* and *Biddulphia sinensis*. Beyond the north of the Isle of Man the flow continues westward and northward into the North Channel. Previous surveys have not extended into the North Channel.

The present results (Fig. 1) show that at the time of sampling in the autumn of 1954 there was an area of water containing relatively high concentrations of *Biddulphia sinensis*, *Sagitta setosa* and *Isias clavipes* near the north end of the North Channel. The regions of maximum local concentration of *Sagitta setosa* and *Isias clavipes* coincided almost exactly; that of *Biddulphia sinensis* was about 10 miles to the south-west, but the patch of this species overlapped with those of the other two. Altogether the distributions of fifteen species were analysed, but only the three mentioned showed local concentrations in the northern North Channel; as other species were not affected, it is unlikely that the concentrations were formed in the region where they were recorded. These same three species also showed considerable similarity of distribution in a lobe extending westward from the north of the Isle of Man, and their concentrations in this area were similar to those in the patch in the North Channel. From the distributions shown in Fig. 1 it appears that the lobe off the north of the Isle of Man consisted of mixed water from the east of the Island, and it seems probable that the patch in the North Channel was of the same origin. The distributions of *Sagitta setosa* and *Isias clavipes* appear to indicate the path that this body of water had followed from the north of the Isle of Man to the northern North Channel.

The fact that the patch of *Biddulphia sinensis* did not quite coincide with the patches of *Sagitta setosa* and *Isias clavipes* may have been the result of uneven horizontal distribution of the three species in the detached body of water, or it may be related to differences in vertical distribution and indicate some shearing of the body of water.

The results also show a distinct patch of *Isias clavipes* off the south-east of the Isle of Man, but the time intervals in the samples make it impossible to estimate accurately its size and shape. Small numbers of *Centropages typicus* and of cyrtopia larvae of *Nyctiphanes couchii* were also present in this patch but otherwise absent from the Isle of Man area. From previous results (Williamson, 1956) the main source of these three species appearing in this region at this season is likely to be in St George's Channel or farther south.

Seasonal and other long-term variations in the rate of the north-going current through the Irish Sea have been previously suggested, but apart from these the flow has been assumed to be fairly regular (see Bowden, 1955;

Williamson, 1956). The distributions recorded in the present paper would, however, be almost impossible to explain in terms of a steady flow. Reasons have already been given for believing that the body of water distinguished in the North Channel was produced by mixing of waters to the east of the Isle of Man and transport of the mixed water so produced. A steady rate of mixing and a steady flow would have produced a tongue of water whose planktonic character changed gradually through further mixing with increasing distance from the north of the Isle of Man. A temporary increase in the rate of mixing would have produced a corresponding increase in the concentrations of *Biddulphia sinensis* and *Sagitta setosa* and a decrease in *Isias clavipes* in the north-going water, because the former two species are introduced into the north-going water by mixing, while the concentration of *Isias clavipes* is reduced by the same process. Similarly, a temporary decrease in the rate of mixing would have produced a corresponding decrease in the numbers of *Biddulphia sinensis* and *Sagitta setosa* and an increase in the numbers of *Isias clavipes* in the north-going water. Only a sudden and temporary increase in the rate of flow from the Isle of Man area could have produced a marked and simultaneous increase in the numbers of all three species in the north-going water by producing a faster moving body of water which would mix less with surrounding water in a given distance and so maintain its planktonic character for a greater distance. It is suggested that such a 'puff' of water was responsible for the local concentrations of *Biddulphia sinensis*, *Sagitta setosa* and *Isias clavipes* recorded in the North Channel.

The patch off the south-east of the Isle of Man containing *I. clavipes* and smaller numbers of *Centropages typicus* and the larvae of *Nyctiphanes couchii* suggests that the flow into the Isle of Man area from the south may also have been irregular.

Salinity measurements are available for all stations except those sampled on 14 and 22 October (Smed, 1955). These show high salinity water off the south-east of the Isle of Man corresponding with the area of greatest abundance of *Isias clavipes*, but they show no patches of high salinity water in the North Channel. As the north-flowing water undergoes sufficient mixing off the Isle of Man to alter drastically its planktonic character, it is not surprising that it should also lose its characteristically high salinity in this region.

On existing information it is not possible to say whether the flow through either the Irish Sea or the North Channel is commonly intermittent. Previous plankton surveys in the Irish Sea (Williamson, 1952, 1956) have given no indication of intermittent flow, but the coverage of the area has never been sufficiently complete to rule out the possibility. Also the sampling area has not previously included the North Channel, and it seems possible that the flow through the North Channel may be intermittent at times even when the flow into the Irish Sea from the south is regular. This could result from a period of south-westerly winds causing an accumulation of water in the Solway Firth region, followed by the release of the water on a reduction in strength or a change in direction of the wind. (Winds were generally south-westerly throughout September 1954; Smed, 1955.) It may also be relevant that the region off the north-east of the Isle of Man consists of shallow water (much of it less than 10 fathoms, 18 m) and is the meeting place of flood tides from both north and south. These two factors, however, are probably more important in promoting mixing than as temporary barriers to the non-tidal flow.

It is not known how common the occurrence may be, but it is improbable that conditions at the time of the 1954 autumn survey were unique. It seems probable, therefore, that the northward flow through the North Channel may at times consist of comparatively fast-moving pulses of water separated by periods of reduced flow. Under these conditions, each pulse of Irish Sea water seems likely to penetrate much farther north before losing its identity through mixing than the slow average rate of flow would suggest. Bowden (1955) has estimated the mean flow across a section at the southern end of the North Channel to be about ¼ mile (400 m) per day, although this figure is likely to be exceeded in parts of the section.

The Irish Sea has been suggested as the source of populations of *Sagitta setosa* recorded in upper Loch Fyne in the Clyde Sea area (Barnes, 1950) and off the Scottish west coast as far north as the Minches (Fraser, 1952). The first of these examples implies the transport of Irish Sea water through about 120 miles (145 km), of which the last 30 miles (48 km) are a narrow cul-de-sac; the second implies the transport of the water through more than 200 miles (320 km). In both cases the Irish Sea origin of the water seems highly improbable if the rate of transport were only of the order of Bowden's figure (see above), but much more probable if the water were transported in pulses travelling at many times this rate.

SUMMARY

The distributions of *Biddulphia sinensis*, *Sagitta setosa* and *Isias clavipes* in late September and early October 1954 suggest that the flow through the Irish Sea and North Channel was intermittent at that period.

Some effects of intermittent flow on the transport of Irish Sea water are discussed.

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TWO NEW MARINE SPECIES OF *GYMNODINIUM* ISOLATED FROM THE PLYMOUTH AREA

By Dorothy Ballantine
The Plymouth Laboratory

(Text-figs. 1–17)

The two new species, *Gymnodinium vitiligo* and *G. veneficum*, which are described below, are both small and highly motile, and in other respects very similar. The greatest difference between them is physiological, as *G. vitiligo* is harmless, whereas *G. veneficum* produces a very powerful toxin which is lethal to fish. There are also small morphological differences which are visible when the organisms are in culture, confirming the existence of two separate species.

I wish to thank Dr Mary Parke of this Laboratory for giving me these two organisms which she isolated, and for her unfailing help and advice, and Dr J. E. Morton, of Queen Mary College, for translating the English diagnoses into Latin.

SPECIFIC DESCRIPTIONS

Gymnodinium vitiligo n.sp. (Figs. 1–5)

Diagnosis

Cells broadly oval, small, not flattened. Epicone slightly smaller than the hypocone, cell rounded at the apex and antapex. Girdle deeply impressed, narrower on the ventral side, displaced about two girdle widths. Anterior pore at the junction of the left side of the girdle and sulcus, posterior pore at the junction of the right side of the girdle and sulcus. Sulcus extends on to epicone and hypocone, deeply impressed at the junction with the girdle and gradually decreasing in depth towards apex and antapex. Transverse flagellum extending all around the body, longitudinal flagellum very fine, 1–$1\frac{1}{4}$ times the cell length. The right ventral side of the epicone is produced into a hook-like flap which protrudes and overlaps the girdle and sulcus to some extent. Nucleus median, indistinct except just prior to cell division. Cytoplasm hyaline, containing refractile oily bodies. Periplast rigid, but without apparent structure, cell slightly metabolic. Chromatophores golden brown, irregular in shape, usually four (varying from 2 to 8 and occasionally more). Cell size 7–$18\mu \times 7$–14μ, most common size $11{\cdot}5 \times 9{\cdot}5\mu$. Not toxic to fish or other animals.

Isolated by Dr Mary Parke from a sample of sea water from 4 fathoms from the Knap Buoy region off Plymouth Sound (lat. N. 50° 19′ 30″, long. W. 04° 10′) on 8 June 1949. Type culture (Plymouth collection no. 102) at the Marine Biological Assocation, Plymouth.

Cellula late ovata, parva, non planata, rotundata ad extremitates apicalem et antapicalem; parte epiconica parum minore quam hypoconica. Cingulo profunde impresso, angustiore ad aspectum ventralem, summoto fere bis suum latitudinem. Foraminibus ad conjunctionem utriusque partis cinguli cum sulco, anteriore sinistrae, posteriore dextrae. Sulco extendente in partem epiconicam necnon hypoconicam, profunde impresso ad conjunctionem cingulo, paulatim altitudine decrescente ut accedit ad extremitates apicalem et antapicalem. Altero flagello transverso totam cellulam circumstante, altero longitudinali tenuissimo, longiore 1–1·25 quam cellula. Dextro latere partis epiconicae producto ex aspectu ventrali in processum fere hamuliformem sed et rotundatum qui super cingulum sulcumque nonnullomodo extendit. Nucleo medio, inconspicuo nisi ante fissionem cellulae. Cytoplasmate hyalino, abundante corporibus oleacibus refractilibus; periplasto rigido, clara structura defecta; cellulae forma paulum mutante; chromatophoris aureo-brunneis, forma irregularibus, ex norma 4 (numero variantibus 2–8, nonnunquam pluribus). Cellulae magnitudine 7–18 μ × 7–14 μ, plerumque 11·5 × 9·5 μ. Non toxica piscibus et ceteris animalibus.

Isolata a Maria Parke exemplo aquae marinae ad regionem Knap Buoy prope Plymouth Sound, profound. *c.* 8 metr., 8 jun. 1949. Cultura typica conservata (Collectio Plymouth num. 102) ad laboratorium Marine Biological Association, Plymouth.

Gymnodinium veneficum n.sp. (Figs. 6–17)

Diagnosis

Cell ovoid, small, not flattened. Epicone and hypocone equal, but epicone more pointed. Girdle deeply impressed, displaced about one girdle width. Anterior pore at the junction of the left side of the girdle and sulcus, posterior pore at the junction of the right side of the girdle and sulcus. Sulcus extends on to the epicone and to the antapex, not deeply impressed, gradually decreasing in depth towards the apex and antapex. Transverse flagellum extending all round body, longitudinal flagellum fine, 1½ times the cell length. Nucleus median, indistinct except just prior to cell division. Cytoplasm hyaline, containing numerous refractile oily bodies. Periplast rigid, but without apparent structure, cell slightly metabolic. Chromatophores golden brown, irregular in shape, varying in number from 2 to 8, usually 4. Cell size 9–18 × 7–14 μ, most common size 12 × 9·5 μ. This species produces an exotoxin which is lethal to fish and other animals.

Isolated by Dr Mary Parke from a sea-water sample from the Hamoaze, over Rubble Bank, off King William Point, South Yard, Devonport (lat. N. 50° 21′ 50″, long. W. 04° 10′ 55″) on 23 June 1950. Type culture (Plymouth collection no. 103) deposited with the Type Culture Collection, Cambridge, and the Marine Biological Association, Plymouth.

Cellula ovoidali, parva, non planata, partibus epiconica et hypoconica aequalibus, epiconica tamen acutiore; cingulo profunde impresso, summoto fere aeque latitudini suo. Foraminibus ad conjunctionem utriusque partis cinguli cum sulco, anteriore sinistrae, posteriore dextrae. Sulco extendente in partem epiconicam et ad extremitatem antapicalem, non profunde impresso, paulatim altitudine decrescente ut accedit ad extremitates apicalem et antapicalem. Altero flagello transverso totam cellulam circumstante, altero longitudinali et tenui, longiore 1·5 quam cellula. Nucleo medio, inconspicuo nisi ante fissionem cellulae; cytoplasmate hyalino, abundante numerosis corporibus refractilibus oleacibus; periplasto rigido, clara structura defecta, cellulae forma paulum mutante. Chromatophoris aureo-brunneis, forma irregularibus, numerosis 2–8, ex norma 4. Cellulae magnitudine 9–18 × 7–14 μ, plerumque 12 × 9·5 μ Cellula substantiam exotoxicam producente quae letalis sic piscibus et ceteris animalibus.

Isolata a Maria Parke exemplum aquae marinae ex Hamoaze, supra Rubble Bank, prope King William Point, South Yard, Devonport 23 jun. 1950. Cultura typica deposita in collectionem culturarum typicarum Cantabrigiensem, sed et ad laboratorium Marine Biological Association, Plymouth (Collectio Plymouth num. 103).

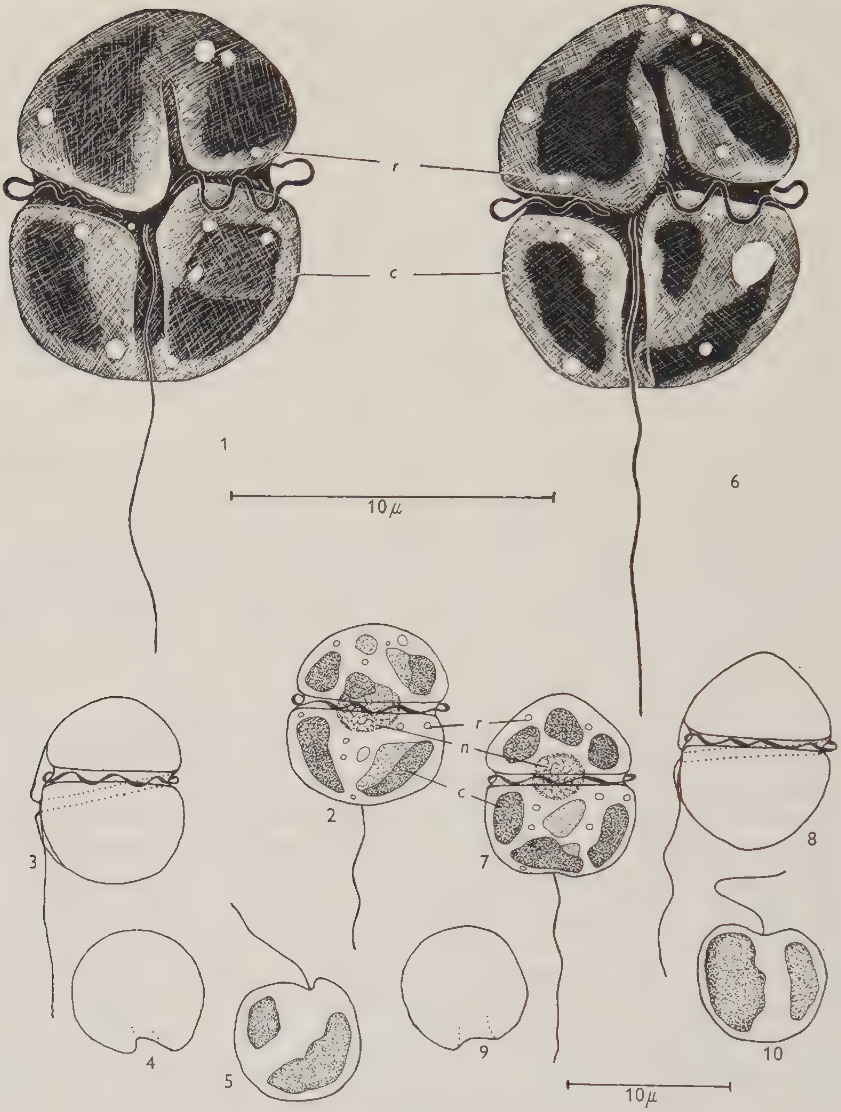
Description

Owing to their similarity the two organisms are best treated together. The features they share in common are described first, and the small but critical differences between them can then be made clear.

Their obvious similarity extends beyond mere superficial resemblances. The absorption spectra of total pigments, obtained from methanol extracts in a Unicam spectrophotometer, are identical. Both show peaks at 444 and 673 mμ, with suggested peaks for pigments other than chlorophyll *a* at 425–430, 460–470 and 620–630 mμ.

Reproduction is by fission in the motile state, and no encystment or sexual reproduction has been observed. Cell division is the same in both species and will be described later for *G. veneficum*. The process appears to take 2–3 h to complete. The final number of cells attained in culture in Erdschreiber in both species is about the same (*ca.* 200,000 cells per ml., exceptionally 500,000 cells per ml.). Colourless cells have been observed rarely in both species, but these appear to occur only in old cultures, and are produced when an organism divides leaving all the chromatophores in one individual (cf. Parke, Manton & Clarke, 1955, p. 592). These cells have not been seen undergoing another division and their rarity in cultures suggests that they die out. Ingestion has not been observed, though both species have been given a large variety of possible foods, both natural and artificial. The chromatophores are irregular in shape, usually plate- or ribbon-like and usually four in number. In old cultures, however, the chromatophores may split up giving an almost reticulate appearance.

These organisms move in a characteristic fashion. They swim forward in a jerky irregular spiral, usually with the ventral side downwards. Movement is quite rapid and the cell rotates from time to time. The transverse flagellum undulates rapidly in the girdle during movement and the longitudinal flagellum either vibrates rapidly (during active swimming) or remains almost still, trailing behind (sluggish movement).



Figs. 1–5. *Gymnodinium vitiligo.*

Fig. 1. Ventral view. Fig. 2. Dorsal view. Fig. 3. View from the left side, showing the displacement of the girdle and projection of the right side of the epicone. Fig. 4. Apical view, showing sulcus. Fig. 5. Antapical view, showing sulcus and longitudinal flagellum.

Figs. 6–10. *Gymnodinium veneficum.*

Fig. 6. Ventral view. Fig. 7. Dorsal view. Fig. 8. View from the left side, showing displacement of the girdle. Fig. 9. Apical view, showing sulcus. Fig. 10. Antapical view, showing sulcus and longitudinal flagellum. *c*, chromatophore; *n*, nucleus; *r*, refractile bodies.

Satisfactory fixation of these naked dinoflagellates has been found to be impossible. They lose shape or burst in many fixatives, and become absolutely unrecognizable in formalin. The greatest success has been obtained with the vapour from 4% osmic acid, but only small quantities of material can be fixed at one time, and even then many cells round off and throw off their flagella. This means that almost all observations have to be made on living material. Both vital staining and staining after osmic acid fixation have been used to try to determine the position of the nucleus and the composition of the refractile bodies present in both species. These bodies appear to be composed of a variety of substances. Starch appears to be formed in very small quantities by some, but not all, individuals in culture, as occasionally small scattered bodies staining blue-black with dilute iodine are observed. After prolonged contact with iodine the whole cell becomes brown. The remaining, usually very numerous, refractile bodies, which are scattered throughout the cytoplasm show up as orthochromatic granules, or vacuoles, absorbing cresyl blue and neutral red without altering the colour of the stain. They also stain with methylene blue and Nile blue. It is felt that these bodies have a lipid base, as osmic acid and Sudan black B both give a black stain. The periplast shows up with trypan blue or iodine staining, but no structure can be seen in either species. *Gymnodinium vitiligo* appears to have a firmer periplast than *G. veneficum*, as it stays a better shape on fixation and survives longer when examined living under the microscope.

In neither species has a pusule been observed, this being either lacking, or, possibly, obscured by the many other cell contents.

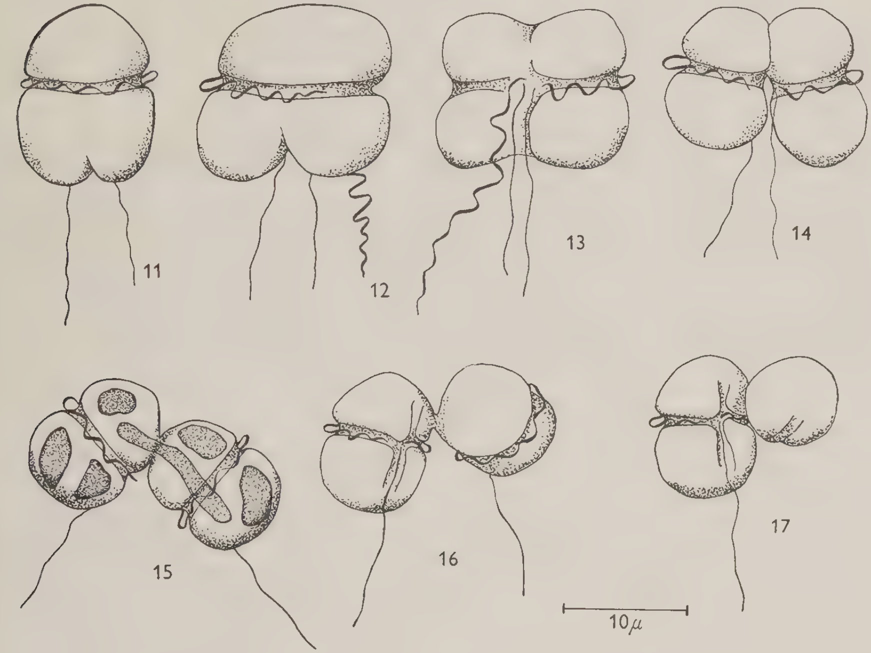
The characters which distinguish these two species are few, and undoubtedly the most important is the toxicity of *G. veneficum*. Whereas all marine animals so far tested have lived for days in *G. vitiligo* culture, fish are killed within a short time by *G. veneficum* (10 min for *Gobius ruthensparri*). This toxicity also affects molluscs, which die in a few hours, and to a lesser extent arthropods and echinoderms. This work will be discussed separately and more fully, in collaboration with Dr B. C. Abbott. The slow toxicity to animals other than fish has already been reported by Bainbridge (1953, p. 393—*Hemimysis lamornae*), Marshall & Orr (1955, p. 510—*Calanus*) and Ballantine & Morton (1956, p. 246—*Lasaea rubra*).

The other distinguishing characters between the two species are morphological and can be seen by comparing the figures (1–10). In general outline *G. vitiligo* is more rounded at the apex (Figs. 1 and 6), has a more deeply impressed sulcus (Figs. 1, 4, 5 and 6, 9, 10) and more displaced girdle (Figs. 3, 8) than *G. veneficum*. The greatest distinction between them under the microscope is the prominence of the hook-like right side of the epicone in *G. vitiligo*. This structure is at times very pronounced, and even if not very well developed shows up the girdle and sulcus very clearly when the cell is living. By contrast there is no protrusion in *G. veneficum*, and the sulcus and girdle are not so clearly defined, nor so deep. The shape of the sulcus also differs when seen in section, as in Figs. 4 and 5, 9 and 10. These views from the apex and antapex show the influence on the sulcus shape of the hook on the right side of the epicone and the less prominent outward development of the left side of the hypocone of *G. vitiligo* as compared with the almost equal development at each side of the sulcus in *G. veneficum*. In addition to these features the longitudinal flagellum of *G. veneficum* is longer and coarser.

With regard to the relationships of these two species within the genus, little can be said. The nearest species appear to be *G. simplex* (Lohmann) Kofoid & Swezy, *G. pygmaeum* Lebour, *G. marylandicum* Thompson and *G. mirum* Ütermohl. *G. brevis* Davis (1948), which is also toxic, differs greatly morphologically, being lobed and flattened, and the remaining species listed all have quite marked distinguishing characters. *G. simplex* has a straight girdle, a sulcus that does not extend to the epicone (Lebour, 1925) and much smaller cells (1·7–7 μ). The sulcus in *G. pygmaeum* Lebour (1925) extends over both apex and antapex and is overlapped on the left side by a tongue-like process, absent in the new species. The remaining two species are freshwater. In *G. mirum* Ütermohl (= *G. rotundatum* Schiller non Klebs and *G. obesum* Schiller, 1933, see Nygaard, 1949) the general shape is similar, but the girdle is straight or only very slightly displaced, the sulcus is very oblique on the epicone and the chromatophores are numerous and small. *G. marylandicum* Thompson (1947) somewhat resembles *G. veneficum* and *G. vitiligo* from the ventral aspect, but differs in being dorso-ventrally flattened. Thus apparently there are no very closely related species in the genus, which though large is very diverse.

NOTES ON CELL DIVISION

Cell division was observed closely in both species, is the same in each, and has been illustrated for *Gymnodinium veneficum* (Figs. 11–17). The first stage of division is observed as a doubling of the longitudinal flagellum and indentation of the antapex (Fig. 11). Shortly after this stage the organism becomes somewhat quiescent. Indentation of the hypocone continues and the new transverse flagellum appears, one remaining in the girdle and the new flagellum free and undulating, usually directed towards the hypocone (Figs. 12, 13). When division of the cell has reached a point above the girdle the new transverse flagellum encircles the body and goes into position in the girdle (Fig. 14). Division of the epicone now proceeds from all directions, until only a thin connexion of cytoplasm is left (Fig. 15). Division of the nucleus is complete at this stage, but nuclear division has not been observed at all stages as the nucleus, though large and median in position, does not show clearly except just prior to division. It was found impossible after fixation to ascertain the stage to which division had proceeded, and thus alcoholic stains could not be used. Normally there are four chromatophores, but an early stage after division may contain only two, which later divide. The chromatophores may also divide prior to, or during, cell division, and organisms giving the appearance of Fig. 15 are not uncommon. The joined individuals are by now again rotating rapidly and irregularly, and during this gyration they pivot about the connecting strand until they are lying in planes at right angles (Figs. 16, 17). They then part, probably as a result of this jerky movement breaking the connecting strand.



Figs. 11–17. Cell division in *Gymnodinium veneficum*.

Fig. 11. Early stage, two longitudinal flagella and divided antapex. Fig. 12. Dorsal view, both flagella duplicated. Fig. 13. Slightly later stage than 12, ventral view. Fig. 14. Hypocones completely separate. New transverse flagellum is now in the girdle. Figs. 15–17. Two new individuals still joined, but twisting round prior to separating. Fig. 15 shows chromatophores in a pair at this stage.

Summary

Two new species of dinoflagellate, *Gymnodinium vitiligo* and *G. veneficum*, have been described and the toxicity of *G. veneficum* is discussed briefly. Notes and figures on cell division in these organisms are also included.

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ON A PELAGIC PENAEID PRAWN, *FUNCHALIA WOODWARDI* JOHNSON, NEW TO THE BRITISH FAUNA

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British Museum (Natural History), London

(Text-fig. 1)

On 30 July 1955 Dr J. H. Fraser, of the Scottish Home Department, Marine Laboratory, Aberdeen, sent to the British Museum for determination two adult females of a penaeid prawn collected at 01.56–02.0 h B.S.T. near the surface (15 fm.) at Rosemary Knoll, to the north-west of the Outer Hebrides, on 22 July 1955. The actual depth at this locality, 59° 12′ N., 10° 09′ W., is 229 fm (419 m). These specimens were identified by one of us (R. W. I.) as *Funchalia woodwardi* Johnson. Later the senior author confirmed this determination and wrote to Dr Fraser as follows:

> This is the first British record of the genus *Funchalia*...For some reason not understood adult specimens of *Funchalia* are rarely captured, unless by fish....In 1936 Burkenroad showed that two species had been confused under *Funchalia* (*Funchalia*) *woodwardi* Johnson and in 1940 he added two new species in the *Dana* collection, one from the Indian Ocean, the other from the Canaries. The older references do not, therefore, necessarily refer to *woodwardi*. Authentic adult specimens of *F. woodwardi* are known from Madeira (holotype, ♀ in B.M.); off South Africa (Calman, 1925, 3 ♀♀, 2 in B.M.; Barnard, 1950, 16 ♂♂, 6 ♀♀, all from stomachs of stock-fish, *Merluccius*); and off Nice in the S. of France (2 ♀♀ in Villefranche-sur-mer Colln. Roger, 1938).

She also asked to see the third specimen, which proved to be an adult male. Dr Fraser's reply was interesting:

> I had no idea, when we caught them [*Funchalia*], that they would turn out to be anything so unusual. We only retained three, out of a total of 84 in a half-hour haul, thinking a quick glance through the literature at Aberdeen would tell us that they were something common enough. (I must confess the remainder of the catch went to prove their comestible value!) Indeed it seems as though they are common enough, given the right type of gear to catch them and used in the right place. Our programme in July was specifically arranged to look into the identification of traces made on the echo sounder and these prawns were the cause of one of them....The gear was an Icelandic Pelagic Trawl specially fitted with a small mesh cod-end (the net designed for catching herring), and I expect we are the first to use such a net near the surface over these oceanic banks.
>
> I do not know yet for certain if they are subject to diurnal migration, but I strongly suspect they are distributed in the deeper layers during the day, where they are caught by the deeper living fish, such as *Merluccius*, and swarm at the surface at night as we found them. [Letter to I. G. dated 17 November 1955.]

Later in September Dr Fraser sent a photo copy of the appropriate part of the echo trace.

> It starts at 00.40 h on 21/vii/55 with a distinct double trace, at 15–20 fm. and at 35–40 fm. approximately, and we chose to sample the lower one first. This gave a haul of small fish only—*Gadus poutassou* and scopelids, mostly *Myctophum glaciale*. By the time we had completed the first haul the two traces had merged—at about midnight (01.40 h B.S.T. at that longitude)—and our second haul thus sampled the mixture. This caught similar numbers of *G. poutassou* and scopelids, and in addition 84 *Funchalia*, so that it seems justifiable to infer that the upper trace prior to midnight was *Funchalia*. This was first seen to appear as a scattered trace at about 23.00 h the previous evening, i.e. about 2½ h before midnight, either because we steamed into the shoal at that time, or because the prawns had started shoaling in the upper layers.
>
> The colour, in life, of the specimens was a pleasant yellowish-orange with a very bright scarlet area (still to be seen in the preserved specimen, though not so brilliantly) on the back of the cephalothorax. [Letter to I. G. dated 26 November 1955.]

Funchalia Johnson

For references and synonymy see *Burkenroad*, 1936, p. 126; *Barnard*, 1950, p. 608.

At present the genus *Funchalia* comprises five species, namely *F. woodwardi* Johnson, 1867, *F. villosa* (Bouvier, 1905), *F. danae* Burkenroad, 1940, *F. taaningi* Burkenroad, 1940, and *F. balboae* (Faxon, 1893). Burkenroad (1934, pp. 76–7, and 1936, p. 136) thought it necessary to establish a new subgenus *Pelagopenaeus* for the last-named species, which differs in several minor details from the first two species. But this difference breaks down, at least in part, since the thelycum of his species *Funchalia danae* resembles that of *Pelagopenaeus*, and as moreover there are so few species in the genus, it seems to us that subgenera are unnecessary.

For many years all specimens of *Funchalia*, whether adult or immature, were referred to *F. woodwardi*, but, as stated in the letter to Dr Fraser, Burkenroad (1936, pp. 128–35) found that two species had been confused under that name. Roger (1938, p. 25) arrived independently at the same conclusion, for he writes: 'Il semble bien que le genre *Funchalia* d'après les auteurs présente au moins deux espèces différentes actuellement connues. *Funchalia vanhöffeni*, que Lenz et Strunck ont décrit, différait assez notablement de *Funchalia woodwardi* Johnson'. Roger was apparently unaware of Burkenroad's paper when he described the two females of *F. woodwardi* in the Villefranche-sur-mer collection. Burkenroad used Bouvier's name *Hemipenaeopsis villosus*, later retracted in favour of *Funchalia woodwardi*, for this second species. Roger's record of *F. woodwardi* from the Mediterranean would seem to confirm Burkenroad's suggestion that larvae referred to *Aristeus antennatus* by Monticelli and Lo Bianco (1902) and to *Aristeomorpha foliacea* by Stephensen (1923) are probably referable to *Funchalia woodwardi*. Where no information has been given as to the number of rostral spines and other characters, or where the material is immature, it is probable that either

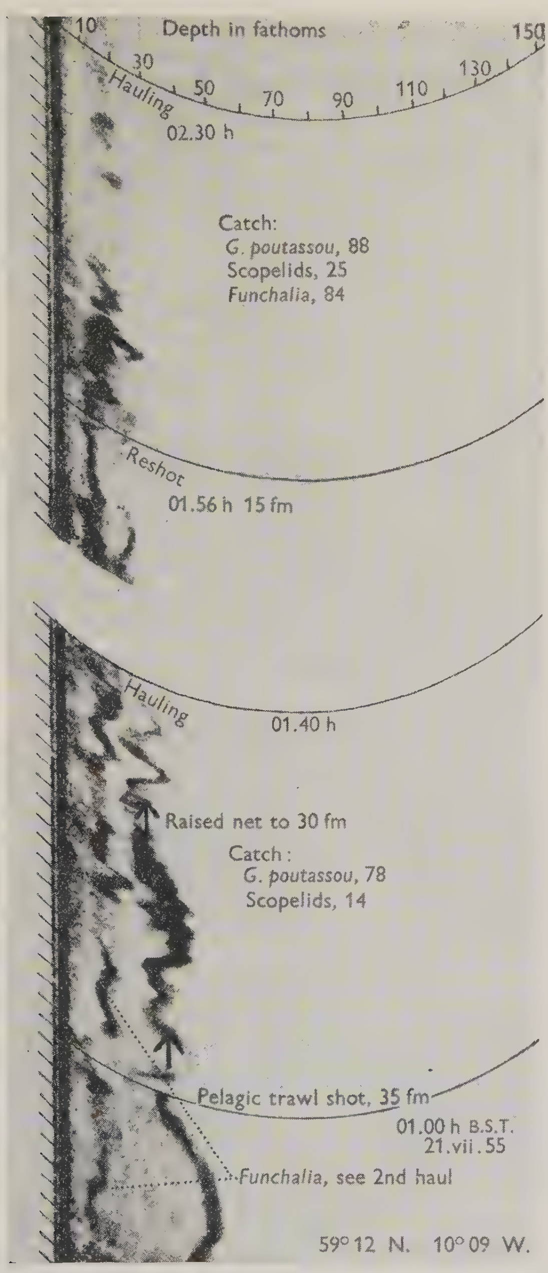

Fig. 1. Echo trace prepared by Dr Fraser. The actual depth is 229 fm and the bottom does not therefore appear on this trace, phased at 0–160 fm. The time scale on left-hand edge is in 2 min ticks. The time at the start and finish of each of the two hauls is given in B.S.T. and the natural midnight at 10° W. is at 01.40 hr. The first catch comprised 78 *Gadus poutassou* and 14 scopelids; the second 88 *G. poutassou*, 25 scopelids and 84 *Funchalia woodwardi* Johnson.

F. woodwardi, or *F. villosa*, or both, are represented. Part of the material may even be referable to *F. danae* which also has a similar rostral formula. One of us (I.G.) has examined the three small specimens, the largest an immature male, from the *John Murray* collection referred to *F. woodwardi* by Ramadan (1938, p. 63, fig. 9). The specimens are imperfect and Ramadan apparently removed not only the left mandible from the largest specimen, but also both mandibles from the small specimens. The mandibles of the two small specimens are now lost, but those of the largest remain. They show a slight asymmetry, such as Burkenroad has noted in other specimens of *Funchalia*, in the distal segment of the mandibular palp. In the left mandibular palp the inner half of the anterior margin extends far beyond the shallow emargination. This is not the case in *F. woodwardi* (Calman, 1925, pl. 3, fig. 7; Roger, 1938, p. 26, fig. 1), but holds good for *F. danae* Burkenroad (1940, p. 36), although not mentioned in the short preliminary note. However, we have examined two rather small females in the *Discovery* collection from St. 1594, 04° 15′ 9″ N., 12° 58′ 2″ W.; these must have been determined as *F. danae* by Burkenroad when he visited the British Museum in 1938–39. It is highly probable, therefore, that Ramadan's specimens and perhaps that of Balss (1925, p. 227) from the Indian Ocean may belong to *F. danae*.

Below we have listed all the references to *F. woodwardi* known to us indicating whether they are authentic, probable, or uncertain, records.

Funchalia woodwardi Johnson

Authentic records

Funchalia woodwardi Johnson, 1867, p. 895. 1 ♀, holotype in B.M. Locality Madeira.

Funchalia woodwardi, Miers, 1878, p. 309 (reference to holotype).

Funchalia woodwardi, Bouvier, 1905, part (acc. to Burkenroad).

Funchalia woodwardi, Bouvier, 1907 and 1908, p. 93, part. For example ♂ from St. 1856, $l = 56$ mm., pl. XV, figs. 1–2; *Grimaldiella richardi* (post-larval) in part.

Funchalia woodwardi, Bouvier, 1922, p. 13, part—♂ from St. 3028, $l = 100$ mm. and perhaps others.

Funchalia woodwardi, Calman, 1925, p. 10, pl. 2, fig. 5, pl. 3, figs. 6–8. 3 ♀♀ from off Cape of Good Hope (2 in B.M.).

Funchalia (*Funchalia*) *woodwardi*, Burkenroad, 1936, pp. 129–35. No new records; compared with *F. villosa* (Bouvier).

Funchalia woodwardi, Roger, 1938, pp. 23–32, figs. 1–4. 2 ♀♀ from Western Mediterranean.

Funchalia (*Funchalia*) *woodwardi*, Barnard, 1950, p. 609, fig. 112*a–h*. 16 ♂♂, 6 ♀♀, from stomachs of *Merluccius* caught Jan.–Feb. 1943, off Table Bay. (3 ♀♀, 6 ♂♂ presented to B.M., 1955.)

Probable records

Aristeus antennatus, Monticelli & Lo Bianco, 1902, p. 30. Probable larvae of *F. woodwardi* acc. Burkenroad, 1936, p. 135.

Aristeomorpha foliacea, Stephensen, 1923, p. 15, fig. 6. Probable larvae of *F. woodwardi* acc. Burkenroad, 1936, p. 135.

? Larva of *Funchalia woodwardi*, Stephensen, 1923, p. 26, fig. 10.

Uncertain records

(*a*) Atlantic Ocean

Funchalia woodwardi, Murray & Hjort, 1912, p. 668 in Table.

Funchalia woodwardi, Sund, 1920, p. 32, 67 immature specimens—the majority from night hauls and 45 from between 50 and 150 m depth.

Funchalia woodwardi, Stephensen, 1923, p. 17. 17 specimens—small ♂♂ and ♀♀ from 45 to 70 mm in length, but no details given; the rest young. (8 in 8 hauls at 7 stations in Mediterranean and 9 in 4 hauls at 3 stations in Atlantic.)

Funchalia sp., Lenz & Strunck, 1914, p. 307, fig. 3*a* and *b*. One immature specimen, off St Helena. The number of rostral teeth suggests either *F. woodwardi* or *F. danae*.

(*b*) Indian Ocean

Funchalia woodwardi, Balss, 1914, p. 593; 1925, p. 227. 1♀ about 50 mm long at 16° 8′ S., 97° 14′ E.

Funchalia (*Funchalia*) *woodwardi*, Ramadan, 1938, p. 63, fig. 9. Three young, the largest an immature ♂. Perhaps referable to *F. danae* Burkenroad (I.G.).

REMARKS

Between 1867 and 1938 adult specimens of *Funchalia woodwardi* were captured sporadically in very small numbers (1 to 3) at various localities in the eastern Atlantic, from the Cape region to approximately 40° N., and in the western Mediterranean. Good descriptions of the female have been given by Calman (1925) and by Roger (1938). For the first time a considerable number of adults were obtained in January–February 1943 from the stomachs of *Merluccius* by Mr Rattray of the Low Temperature Laboratories, Cape Town, while he was studying the food and diseases of the stock-fish. Although many of the specimens were mutilated, Barnard was able to describe and figure for the first time the petasma and appendix masculina of the adult male (Barnard, 1950, p. 609, fig. 112*e* and *f*). The petasma is asymmetrical, either the left or the right half being the longer.

The haul mentioned by Dr Fraser in the letters quoted above (see p. 475) is of special interest because for the first time free-swimming adults were obtained in quantity—84 in a half-hour haul. The three specimens selected at random as a sample for determination are a male and two females measuring from 120 to 130 mm in length, neither female being impregnated. The catch, therefore, apparently consisted of adults of both sexes. Moreover, this is the first British record and by far the most northerly locality for the species. The depth at which the adults were swarming, as indicated by the upper trace on the echo sounder, was approximately 15–20 fm or less than 40 m.

Little is known of the bathymetric range, habits or development of *Funchalia*. Bouvier regarded the genus as bathypelagic, though capable of ascending to the upper layers; 'from the catches of the *Michael Sars* it would appear that the young at least are denizens of the upper water layers though not of the very surface' (Sund, 1920, p. 32). Most of the *Michael Sars* specimens were collected in night hauls at 50–150 m, but of the seven taken in day hauls four were also from 50 and 100 m (Murray & Hjort, 1912, table on p. 668)*. Burkenroad says that *Funchalia* is a pelagic genus with autogenous statolith in the statocyst chamber which, however, retains an opening to the exterior (1936, p. 128). Sund regarded all his material as immature, although the largest specimen measured 75 mm in length; Bouvier and Stephensen sexed their specimens of about 45–70 mm but the majority of these, if not all, were probably not fully adult. The maximum recorded length is approximately 157 mm (holotype, rostrum damaged). It is quite likely that *F. woodwardi* may exhibit vertical diurnal migrations of considerable magnitude such as have been described for *Gennadas* and other deep-water Crustacea Malacostraca (Waterman *et al.*, 1939).

The British Museum authorities are indebted to the Director of the South African Museum for nine specimens obtained from stomachs of *Merluccius* and to the Director of the Scottish Home Department, Marine Laboratory, Aberdeen, for permission to retain the male and one female from Rosemary Knoll.

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* This work lists sixty-two specimens, but the total given in Sund, 1920, p. 32, is sixty-seven.

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THE NANSEN CLOSING METHOD WITH VERTICAL PLANKTON NETS

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(Plates I and II and Text-figs. 1 and 2)

Several methods have been devised for sampling plankton in the different water layers in the ocean, but for many years vertically hauled nets closed by a method devised by Nansen (1915) have fulfilled this purpose. Recently, however, an investigation by Barnes (1949) cast doubt on the validity of this method for quantitative work, and as in his paper he specifically referred to nets of the type used on the 'Discovery' Expeditions, the present observations were conducted to determine whether Barnes's findings were of general application to the method.

The Nansen closing principle, which will be described in more detail later, employs a throttling rope which encircles the net some distance behind the mouth, and which becomes the towing line when the front towing bridles are released, and closes the net. Although the method is adaptable to almost any medium-sized net, with vertical hauls it is most frequently used with the Nansen net (Künne, 1929), and in this manner it has been employed extensively. Hjort in the Norwegian plankton investigations (Hjort & Ruud, 1927, footnote, p. 32; Murray & Hjort, 1912, p. 46), the 'Discovery' Expeditions in the Antarctic, the British, Australian and New Zealand Antarctic Research Expedition, the Australian, South African and many other fishery research organizations have all used the method.

More recently, in view of the claims made for the superiority of obliquely hauled nets (mostly on the grounds that they are less liable to be affected by patchiness of the plankton distribution), many workers have tended to replace the vertical net with such devices as the Clarke-Bumpus sampler and other obliquely towed apparatus (Wiborg, 1948). In the collection of zooplankton in deep oceanic waters, however, practical problems arise. Oblique hauls in several thousand metres of water become a tedious and time-consuming operation, whereas vertical hauls can conveniently be carried out at the same time as water-sampling and other such operations. Furthermore, one can be much more certain of fishing a specific depth horizon with a vertical haul.

It is also desirable in plankton collection to use standardized methods as far as is possible. This simplifies greatly the comparison between observations made by different workers, at different times and in different places. It was, therefore, not only with regard to the vast amount of earlier work which has been done with the method, but also in view of future work which is anticipated, that it was felt a matter of some importance to undertake the present observations.

It was originally intended to study the closing of the net in an experimental tank, watching, by means of coloured dyes or streamers, the movement of water in the net during the closing process. It was at once apparent, however, that it would be impossible to simulate in the laboratory the actual working conditions which prevail at sea. The effects of the rolling of the ship and the action of the special davit from which the net is used would be extremely difficult to reproduce in the laboratory. In view of this, it was decided that direct observation of the normal performance of the net at sea would be the only satisfactory way of studying the closing process and then a record could be made of it by means of underwater cine-photographs. Suitable opportunity for doing this occurred in September and October–November of 1955, when the R.R.S. *Discovery II* was working in the region of the Canary Islands. Preliminary observations were first made by swimming with face masks while the net was being operated from the ship, and later, for the underwater cine-photography, the ship went to the Salvage Islands. Here, the water was warm and exceptionally clear, presenting ideal conditions for photography, and it was possible to anchor the ship in the calm water under the lee of the islands.

As far as we are aware these photographs are the first attempt to record the performance of a plankton net under actual working conditions at sea.

BARNES'S OBSERVATIONS

The data which were analysed by Barnes (1949) were obtained by Marshall, Nicholls & Orr (Nicholls, 1933) in their study of the Clyde Sea Area in 1932. The net hauls were taken by means of a modified International net fitted with a Nansen (1915) closing mechanism. The net, according to Nicholls (1933), differed from the standard pattern of Ostenfeld & Jespersen (1924) in that 'the cylindrical part of the net was made of canvas instead of netting and the conical part of fine-meshed bolting-silk (77 strands in 1 cm). An ordinary townet bucket was used. A hand winch was used for hauling the net, the rate at first being kept as nearly as possible to half a metre per second.' Dr Barnes informs us that no accumulator springs were used on the davits.

From a statistical analysis of the composition of the catches of undivided and divided hauls (divided meaning that the net was hauled through a specific depth horizon and then closed), Barnes reached the conclusion that a significant loss of catch took place from the divided haul or, in other words, when the Nansen closing method was brought into use. He illustrated this in particular from the catches of *Microcalanus pygmaeus*, a copepod which was present only *below* the level of closure of the divided haul. Of this organism the catches in the divided hauls were significantly less than those in the undivided hauls.

To determine the cause of this loss from the divided haul, he carried out some experiments with a model net in the mouth of which was hung a bag of powdered potassium permanganate. An electric motor was used to tow the model horizontally in a tank, and closing was simulated by stopping the motor and reducing the forward speed of the model by a line attached to the tail of the net. A colour film taken of those experiments showed that when the net was stopped the water in the net tended to flow out of the mouth of the net. Recently Motoda & Anraku (1955) have observed this to occur with a full-scale net. Thus it seemed that in the full-scale net it must be the deceleration or falling back of the net while it closed that accounted for the loss of catch.

The net which Barnes considered, and a model of which he used in his experiments, he refers to as the 'Discovery' net. However, the measurements show that it is actually a model of the 'Discovery' phytoplankton net, the N50V, a net which is practically never used with the Nansen closing mechanism. Only on a few occasions has the N50V ever been closed, and this only to prevent ripping of the net as it comes out of the water in very bad weather.

The net which is normally used with the Nansen closing mechanism on board the R.R.S. *Discovery II* is the so-called N70V. This net is larger and of much coarser mesh than the N50V, and this fact combined with the method of operation led us to expect that its behaviour might be quite different from the International net, and models of the N50V which Barnes used.

THE 'DISCOVERY' NET (N70V)

This net was based on that used by Hjort (Hjort & Ruud, 1927, p. 32, footnote), and is described by Kemp, Hardy & Mackintosh (1929). It is similar to the Nansen net of Künne (1929). It is 70 cm. in diameter at the mouth and is constructed of four sections, the first three of which are almost cylindrical while the final section tapers conically to the net bucket. The first section is of $\frac{1}{4}$ in. mesh netting, and this is followed by a canvas band bearing six brass rings through which the throttling rope is led. The third section, which is almost cylindrical, is a length of bolting-silk having 40 meshes to the linear inch, and the fourth section which tapers conically to the net bucket is a length of bolting-silk having 74 meshes to the linear inch. The net bucket has on its side a gauze window of 140 meshes to the linear inch. The throttling rope is $16\frac{1}{2}$ ft. long, which allows 4 in. of slack when the net is properly rigged. It is threaded from the release gear down and around the canvas band and back up to the release gear, so that the net when it closes is caught up in a bight of this rope. The net is towed by three bridles, $\frac{1}{4}$ in. brass rods 32 in. long joining into a single ring which fits into the closing mechanism. Below the net is a weight of about 40 lb. supported on three phosphor-bronze stays, which run the full length of the net and are attached both to the net bucket and the net ring.

With the ship hove to, the net is lowered to the required depth on a wire running over a metre counter on a specially designed davit. The latter incorporates a long accumulator spring on a movable sheave (Text-fig. 1). With the rolling of the ship this spring helps to keep an even strain on the wire, preventing any undue slackening as the net is paid out. On reaching the necessary depth, the steam winch is put in gear, and a messenger on a long line is clipped on the wire. Hauling is commenced, and a steady rate of 1 m/sec (compared with $\frac{1}{2}$ m/sec of the International net) can easily be maintained by regulating the steam valve, while a stopwatch is used to check the readings of the metre counter. As the net is hauled upwards, the winch and accumulator spring maintain a tension on the wire to the net which never relaxes, even in the very worst weather conditions. One can be fairly certain, therefore, that the net travels upward at quite a uniform speed. At a predetermined instant the messenger is released from the line—e.g. if it is desired to close the net at 500 m the messenger would be released at a metre counter reading of 660 m, since it travels at 3·12 m/sec and would take 160 sec to reach 500 m—the hauling time required for 160 m. The closing of the net can easily be seen by a sudden drop of the accumulator sheave, and only very rarely is the depth of closure more than 1–2% different from the calculated depth even on a 500 m haul.

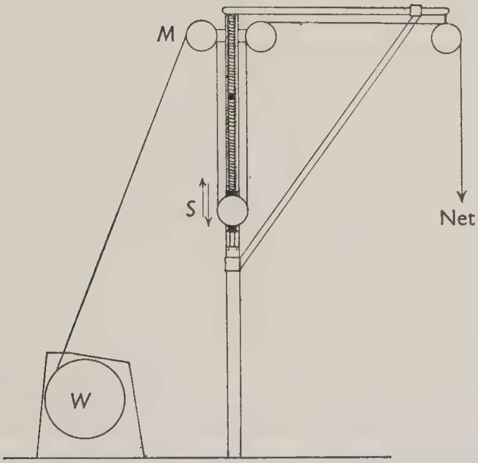
As the messenger hits the release mechanism, the brass towing bridles are released, and the towing point transfers to the throttling rope which closes the net by tightening the bight behind the mouth of the net. When this takes place the accumulator spring suddenly extends, taking in the slack on the wire, while at the same instant the steam winch accelerates momentarily until the wire to the net is once more taut.

The Underwater Observations and Photographs

For the purpose of observing the net closing underwater the ship was anchored in about 15 fathoms of water to the leeward of Selvagem Grande (in September) and Selvagem Pequena (in November). The net was fished from the port forward well-deck winch to facilitate the photography, which was controlled from a rubber dinghy, the swimmers being equipped with face masks and frogmen's feet. In a calm sea and with the ship at anchor, the net-wire led vertically into the water. It was normally paid out to 25 m, at which depth, in the clear waters of the Salvage Islands, it was perfectly visible, and it was thus possible to observe the net throughout the whole of its operation.

To ensure that the net would close just below the surface, two large stream-lined messengers were clipped on the wire and suspended about 1 m below the surface. The bore and weight of the messengers were such that the wire could be hauled through them, so to speak, without any tendency for the messengers to ride up the wire out of the water. The net, on the impact of the release mechanism and messenger, closed in the normal manner. In the hope that they would indicate direction of flow, tapes were attached on cross-wires inside the mouth and at several points further down the net. The movements of the tapes in the mouth were clearly seen (see Pls. I and II).

The attachment of tapes, the suspension of the messengers and the anchoring of the ship were the only deviations from the normal routine method of fishing the N 70 V, none of which could in any way alter the performance of the net. In the course of these investigations some thirty vertical hauls were made in this manner.



Text-fig. 1. Diagrammatic arrangement of sheaves and accumulator spring on davit. *M*, measuring sheave; *S*, sliding sheave; *W*, winch.

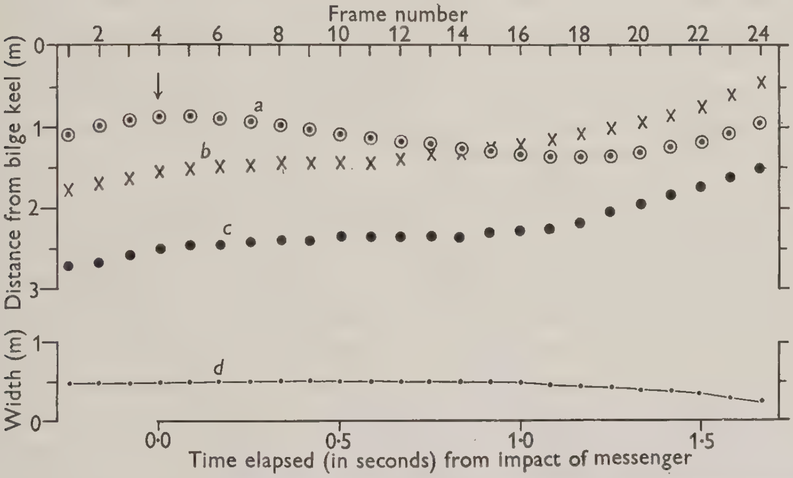
Observations of the net as it is lowered show that both the silk section and the canvas throttling band are collapsed by the pressure of water forcing them up towards the ring, where the tapes can be seen leading upwards. The filtering part of the net is thus constricted, making it virtually impossible for the net to catch plankton on the way down. The shape of the net is, in fact, identical to that figured in Murray & Hjort (1912, fig. 29*a*, p. 46). On hauling, the pressure of water fills out the net. The net rises steadily in the water until the release mechanism strikes the messengers and closing takes place. The bridles are released and the net ring starts to fall in the water; at the same time the net appears to decelerate slightly and the throttling rope tightens, closing the net, which then continues on its upward path. The closing takes but an instant and seeing it for the first time one is immediately struck by the speed and smoothness of the whole operation.

The quickness of the throttling makes it impossible to see anything more than has just been described. Of the hauls that were made, however, some twenty were successfully photographed, including two in which the messenger was released in the normal manner (see p. 486) and allowed to run down the wire to close the net at a predetermined depth—in this case 5 m. The resulting cine-film makes it possible to analyse and measure the sequence of events in greater detail, and Pls. I and II show a series of stills made from a typical section of the film. They are of alternate frames, the time interval between each shot being $\frac{1}{12}$ sec, so that the whole series represents 2·0 sec of the haul.

The first three frames in Pl. I show the net being hauled vertically through the water at a speed of 1 m/sec, with the silk filtering section filled out, and the tapes in the mouth leading down into the net. On the impact of messenger and release gear (frame 4) the bridles are released (frame 5) and the net starts to close. The net ring having some forward momentum does not fall immediately, and throttling is well under way before the tapes start to fall out of the net ring (frame 7). The momentary action of the accumulator spring (p. 486), together with the speeding up of the winch (see p. 486), causes the throttling rope to tighten quickly as can be seen in frames 5–9 where first the slack in the throttling rope is taken up and then the release gear shoots up out of the picture. The throttling rope quickly constricts the canvas band, and the net, which decelerates slightly (frames 6–12), is closed in the less than 1·0 sec (Pl. II, frame 16) after the impact of the messenger.

Of particular note is the fullness of the net behind the canvas band which persists after the release of the bridles. During throttling, the mouth of the silk section is constricted and there must therefore be some decrease in the volume of water inside the net, possibly causing water to be expelled from the upper part of the net. If such a loss does occur it must be very small since any appreciable loss of water would cause the back section of the net to collapse very rapidly. The reverse in fact appears to occur and the action of the throttling rope seems to be to maintain or even increase the internal pressure of water in the net. Even after complete throttling there is still a considerable bulge in the silk which disappears only when the net has once more resumed its upward path through the water (frames 16–24).

The bilge keel of the ship, which is clearly shown in each frame of the sequence, provides a convenient point of reference from which it is possible to measure, and, thereby follow closely, the successive positions of the various parts of the net. Such measurements, taken from Pls. I and II, can be converted into metres using the known dimensions of the net—in this case the diameter of the ring. Text-fig. 2 shows the distances in successive frames from the bilge keel to (*a*) the net ring, (*b*) the throttling point, and (*c*) the end of the first silk section (the white horizontal band in the photographs), together with (*d*) the width of the end of the first silk section. This diagram confirms the previous description of the process of throttling. It will be seen that the filtering part of the net, represented by curves (*b*) and (*c*), does not drop in the water, although it does decelerate and is practically stationary for about a $\frac{1}{4}$ sec (frames 8–11). It then accelerates and by the 15th frame the point of throttling has passed the falling net ring (curve *a*) and the net is then completely throttled. It is only then, as it is once more hauled rapidly through the water, that the bulge in the silk, represented by the width of the white band (curve *d*), starts to decrease. The time scale shows that complete throttling occurs about 0·75–1·0 sec after the impact of the messenger, and similar measurements taken from two other vertical closing hauls selected at random from the film confirm this time.



Text-fig. 2. Measurements taken from Plates I and II, showing the distance from the bilge keel of the ship to various parts of the net, plotted against time. The width of the end of the first silk filtering section is also shown. The arrow indicates the point of impact of the messenger on the release gear. *a*, net ring; *b*, throttling band; *c*, end of first silk section; *d*, width of end of first silk section.

DISCUSSION

To understand the difference in behaviour between the 'Discovery' net (the N70V) and the modified international net used by Barnes, we must take into consideration the different construction and method of operation of the nets.

If a net has a low filtration coefficient, then only a small quantity of water actually passes through the meshes, and the water which does not escape in this way must be carried along by the net, creating a back pressure against the inflow of water at the mouth of the net. As the filtration coefficient increases, so more water escapes through the meshes and the back pressure must reduce until it reaches zero at 100% filtration coefficient.

Suppose now that the net is stopped instantly, there will be a tendency for any back pressure to manifest itself in an outflow of water from the mouth of the net, and this tendency will become greater the lower the filtration coefficient is. It is, therefore, a point of great importance that the N70V with its relatively wide mesh (40–74 meshes/in. compared with 200 meshes/in. of the International net) has a very much higher filtration coefficient than the fine-meshed International net. We have made experiments in which vertical hauls were made with a flow-meter mounted in a net of the N70V type and compared with hauls in which the meter alone was used, and these show that the filtration coefficient of the 'Discovery' net is of the order of 80–90%. Not only does the filtration coefficient depend on the mesh of the net, but also on the ratio of the area of the filtering surface to that of the mouth opening. The filtering part of the International net is a simple cone two metres long, and the area of this relative to the mouth area is about 8 to 1. In the N70, however, the filtering surface can be considered as a cylinder followed by a cone, and in this case the ratio is nearly 10 to 1.

When plankton is caught in a net, it is doubtful just where it accumulates. In nets where a filtering window is used in the bucket (e.g. the N70V) the plankton may well descend into the bucket, but there is quite a lot of evidence suggesting that when a blind, non-filtering bucket is used as in the International net (see p. 484), the plankton may well collect farther up the net. Mr J. W. S. Marr (personal communication) attempted closing the very large $4\frac{1}{2}$ m diameter nets, in which the bucket was non-filtering, at a point just above the bucket. In those cases nearly all the catch was found above the closing line. If this applies to smaller vertical nets, it is clear that should a blind bucket be used on short hauls where the length of water column inside the net is an appreciable fraction of the length of water column fished, then there will certainly be an increased tendency to lose organisms from the catch.

We have emphasized the importance of the accumulator spring and steam winch ensuring the rapid closing of the N70V. Although it is realized that the human arm in the operation of a hand winch such as used by Nicholls (1933) is a fairly effective accumulator, the reaction time of a strong spring must be considerably less than that of a man's arm released from a great strain, and it is no doubt on account of this, and the great flexibility of the steam winch, that the N70 throttles so rapidly compared with the International net (Barnes, 1949, fig. 6). (It should of course be remembered that the hauling speed of the 'Discovery' net is twice that of the International net.)

One final point that might be mentioned is the form of the closing rope. In the N70, this is a *bight* of rope extending down from the release gear and encircling the net. Some authors (e.g. Ostenfeld & Jespersen, 1924), recommend the use of a single line descending from the release gear and forming a *running noose*, around the net. With this, obviously, there is much more slack to be taken in before the net is throttled, and this method should certainly be avoided.

CONCLUSIONS

The observations which are presented here cannot be regarded as conclusive proof of the infallibility of the Nansen closing method. They do show, however, that it is highly improbable that any significant loss of catch can occur if the method is applied in the way it has been to the 'Discovery' net. The latter differed from the International net in respect of its high filtration coefficient. The other mechanisms which help to avoid loss of catch are the filtering window in the bucket and the rapidity of closure which is effected by the efficient accumulator and the steam engine's capacity to adjust the speed of the winch automatically to the tension of the wire. We would, however, stress that we are in entire agreement with Barnes's findings as applied to the International net as Nicholls used it, and this must be looked upon as a warning against the use of catches, made with the Nansen method, for quantitative work, before ascertaining in what manner the hauls have been made.

It will be obvious that this work would have been impossible without the assistance of all our colleagues on board the R.R.S. *Discovery II* in the September and October–November cruises in 1955. To them we should like to express our gratitude, and in particular to Mr Edgar French who made the majority of the net hauls while the photographs were taken. We are also indebted to the Director and Lieut.-Commander Terrell of H.M. Underwater Countermeasures and Weapons Establishment for the loan and guidance in the use of the underwater camera.

SUMMARY

The object of the work described in this paper was to find out if the Nansen closing method used with vertical net hauls can be reliable in quantitative plankton investigations.

Barnes has shown that the International net, which is a fine-meshed net, can lose (through the mouth) a significant part of the catch at the moment of closure by the Nansen method.

The net used by Barnes, however, is not comparable to the 'Discovery' net which has a much higher filtration coefficient and is used with gear which ensures that there is no relaxation of tension on the towing wire when the net is closed.

Underwater observations and films have demonstrated that closure is very rapid and that there is little doubt that the Nansen closing method can be quite reliable provided certain precautions are observed.

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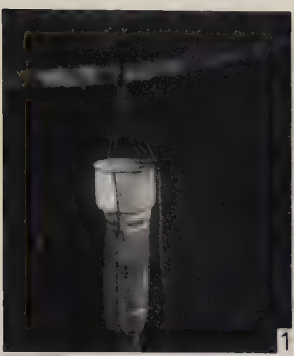
EXPLANATION OF PLATES I AND II

These photographs are alternate frames from a 35 mm cine-film, taken at a speed of 24 frames/sec. The time interval between each photograph is therefore $\frac{1}{12}$ sec. The point of impact of the messenger is on the 4th frame.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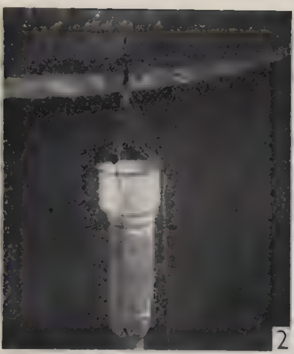
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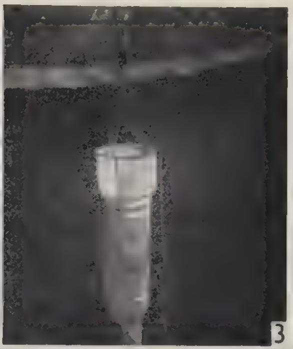
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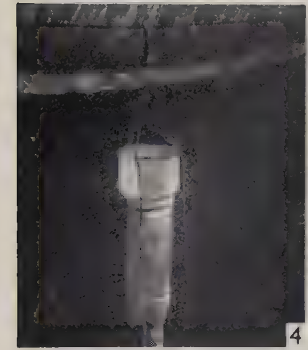
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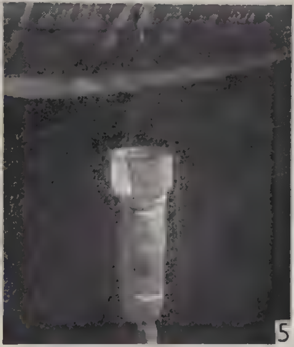
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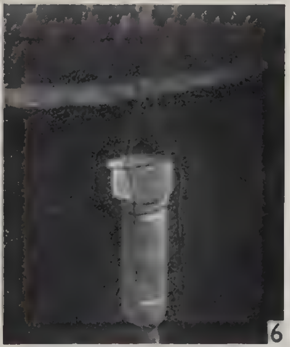
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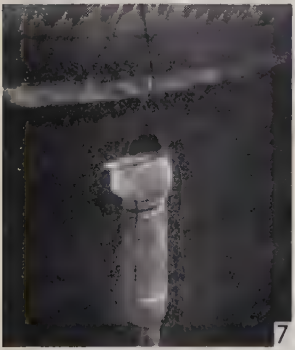
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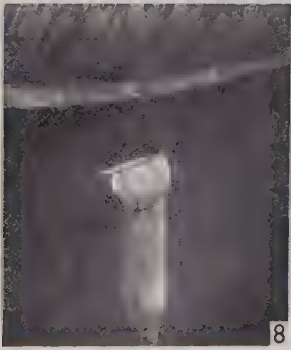
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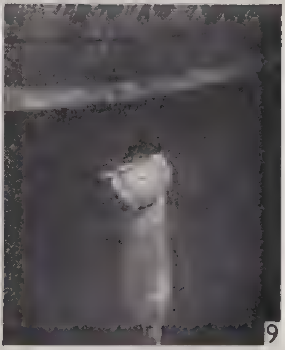
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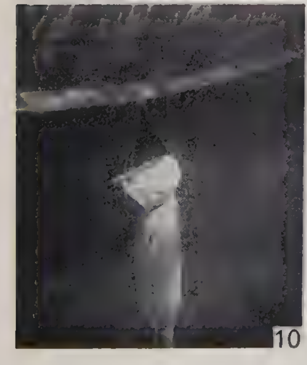
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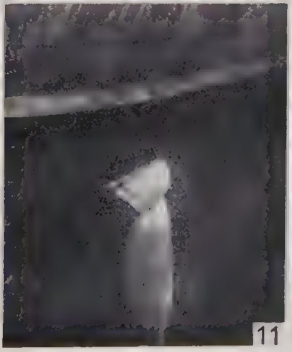
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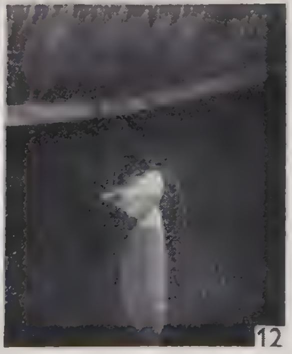
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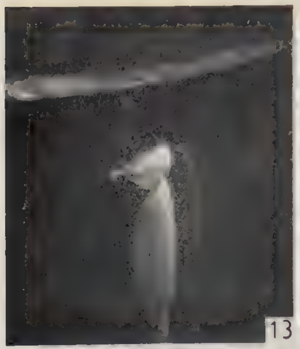
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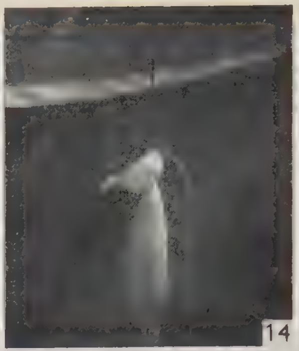
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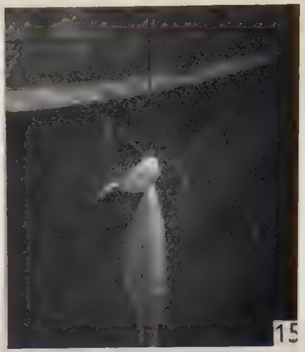
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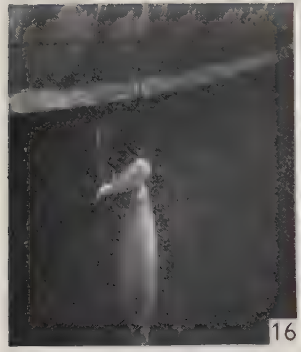
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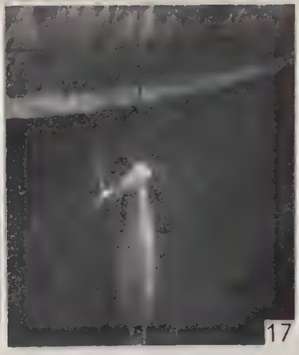
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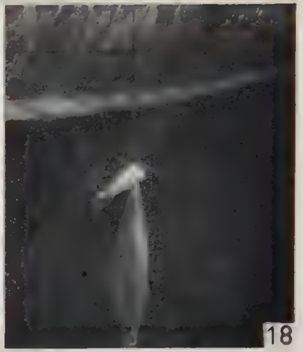
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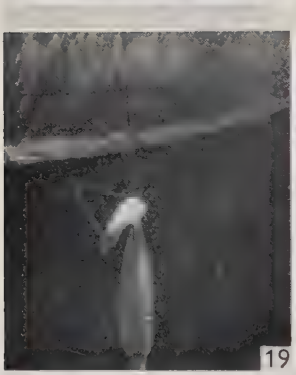
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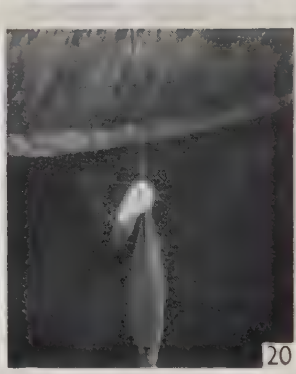
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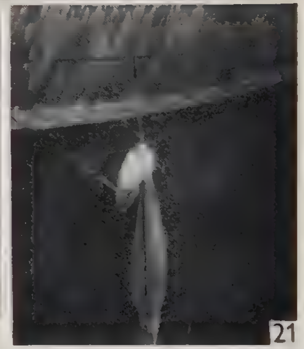
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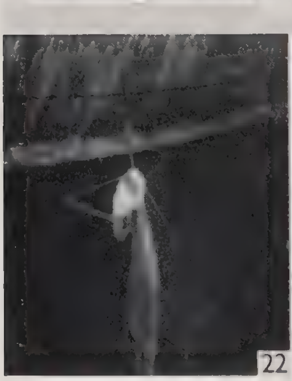
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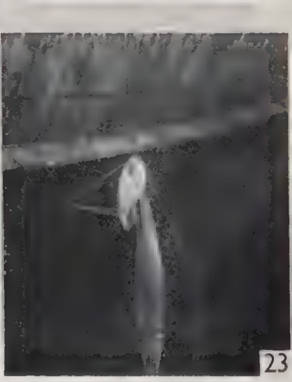
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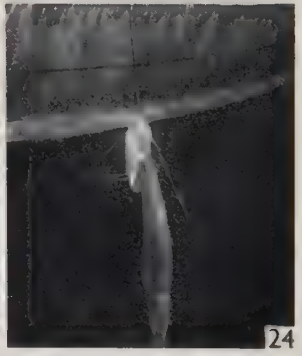
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ON TWO NEW MEDUSAE, *MERGA REESI* N.SP. AND *TIAROPSIDIUM ATLANTICUM* N.SP.

By F. S. Russell, F.R.S.
The Plymouth Laboratory

(Text-figs. 1–3)

In a collection made with a 2 m stramin ring trawl at 47° 12′ N., 7° 40′ W. on 21 July 1955, with 450 fathoms of wire out, I have found two new species of medusae. These were each represented by a single specimen, one of which was an anthomedusa and the other a leptomedusa.

The anthomedusa was much crushed and contracted, but it was nevertheless intact and it has been possible to make a fairly complete description of it. It is apparently a pandeid, but I have had difficulty in placing it in a known genus. It is evident that the classification of some pandeids needs revision, but until more is known it seems premature to attempt this. In the meanwhile I am provisionally placing this new species in the genus *Merga*, as Kramp (1956) has recently done for another new pandeid. I propose to call it *M. reesi* n.sp. after my colleague Dr W. J. Rees, whose researches on hydroids and their medusae are well known.

The medusa, of which a reconstruction is given in Fig. 1, can be described as follows.

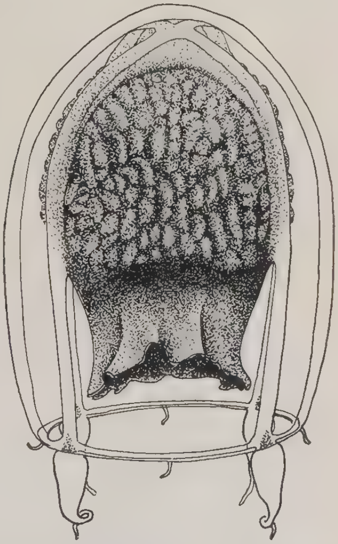

Fig. 1. *Merga reesi* n.sp. Reconstruction from damaged specimen.

Merga reesi n.sp.

Umbrella bell-shaped, higher than wide, no apical process. Stomach flask-shaped, not extending beyond umbrella margin, with broad base. Mouth with four slightly crenulated lips. Four radial canals and ring canal broad, with smooth outlines. Radial canals attached to stomach over more than half its length to form 'mesenteries'. Gonads interradial, irregularly folded to form numerous small raised corrugations. Four perradial marginal tentacles with swollen elongated basal bulbs, without exumbrellar spurs. Four small tentaculae, one in each interradius. No ocelli. Height 10 mm. Colour of stomach and gonads dark chocolate red; basal bulbs of marginal tentacles colourless, except for slight internal pigmentation at junction of radial canal and ring canal.

The specimen has been deposited in the British Museum (Natural History) and has been given the registered number B.M. 1956.4.14.1.

The other specimen was a large leptomedusa. I showed it to Dr P. L. Kramp, when he was in Plymouth, and he immediately identified it as a species of the mitrocomid genus *Tiaropsidium*. The medusa has the distinctive characters typical of that genus.

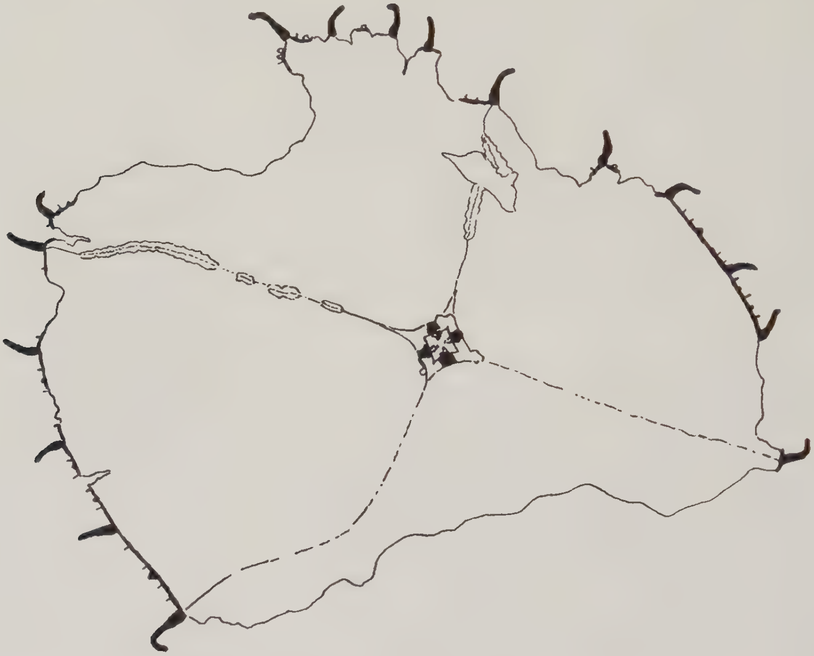

Fig. 2. *Tiaropsidium atlanticum* n.sp. Drawing of specimen showing actual state of preservation.

The specimen is somewhat damaged; the margin of the umbrella is not entire and some of the gonads are missing. Nevertheless, the portions that remain are in a good state of preservation. The umbrella is about 60 mm in diameter and flatter than a hemisphere. The most striking feature is the black pigment on the stomach and on the large marginal tentacles.

The medusa most closely resembles *T. japonicum* Kramp. It differs from that species in its greater size, its higher number of marginal tentacles and vesicles, and the presence of the black pigment.

In outline the stomach resembles exactly the drawing given by Kramp (1932, text-fig. 1) for that of *T. japonicum*, but on each of its interradial walls it has black areas forming four triangles on the base of the stomach each of which is continued as a black streak down each mouth lip (Fig. 3*a*, *b*). The gonads are linear and extend along the middle three-quarters of the radial canals; they have median longitudinal divisions. The specimen is a mature male and the gonads are somewhat follicular in appearance (Fig. 3*d*).

The large marginal tentacles appear to be similar in form to those of *T. japonicum*. Each tentacle has a narrow abaxial and a broader adaxial streak clearly demarcated from the black pigmented lateral areas; these may be indications of a similar arrangement of muscles to that described by Kramp (1932, p. 328) for *T. japonicum*. There is a triangular prolongation of the exumbrella extending a short way down the abaxial side of the base of each tentacle (Fig. 3*c*, *d*).

The medusa has the small tentacles on the umbrella margin characteristic of the genus, and the open marginal vesicles are typical, each having a large black ocellus and containing 12 to 20, or possibly more, concretions.

A drawing of the whole medusa, showing the missing portions of the umbrella margin, is given in Fig. 2. I have counted the marginal organs in those parts of the margin which are complete and the sequence in each quadrant is as follows:

Quadrant	
1	*T t t v t (T) t v t T t v t v t T t – – t v t T t – – – t*
2	*T t – – – – T t v t v v T t v – – – T – v – – – T – – – – – T – – – t t*
3	*T – – – – t T – v – – – T t t (T) t t T t v t v t T – – – – –*
4	*T –*

T = large marginal tentacle; (*T*) = bulb of developing large tentacle; *t* = small marginal tentacle; *v* = marginal vesicle. The dashes indicate missing portions. The first *T* in each quadrant is opposite a radial canal.

It is to be seen that the greatest number of large tentacles in any one quadrant is six, the number in the remaining two complete quadrants being 4 plus 1 developing and 5 plus 1 developing, respectively. A drawing of the first two sections of the first quadrant is given in Fig. 3*c*. In the centre of the first section between two large tentacles (on the left) there is a bulb of a developing large tentacle; this is flanked on either side by two small tentacles with a marginal vesicle between them, and on one side a third small tentacle developing. In the second section (on the right) there are two marginal vesicles each flanked on the outer side by a small tentacle and having a third small tentacle between them. There are evident abnormalities such as two marginal vesicles adjoining one another in the second section of the second quadrant, and no marginal vesicles in the third section of the third quadrant.

It seems reasonable to suppose that the sequences in the second sector of the first quadrant and the fourth sector of the third quadrant represent the final development, that is two marginal vesicles and three small tentacles between two large tentacles. On this basis a fully developed specimen could have

$4 \times 6 = 24$ large tentacles
$24 \times 3 = 72$ small tentacles
$24 \times 2 = 48$ marginal vesicles

In my damaged specimen there are three quadrants complete as regards the large marginal tentacles. The number of large tentacles in these three quadrants was 15 plus 2 developing. If the specimen had been complete it is therefore likely that it would have had at least twenty fully developed large tentacles.

This medusa therefore differs from all known species of the genus *Tiaropsidium* in having a considerably higher number of large marginal tentacles and marginal vesicles, the greatest number so far being 8 tentacles and 16 marginal vesicles, in *T. japonicum*. The combination of higher numerical characters, striking black pigment, and locality of capture supplies sufficient grounds for regarding this specimen as belonging to a new species to which I give the name *T. atlanticum*.

Since this new species may perhaps have as many as 48 marginal vesicles it will be necessary to amend the generic diagnosis given by Kramp (1932, p. 366) accordingly.

The new species may be described as follows:

Tiaropsidium atlanticum n.sp.

Umbrella flatter than a hemisphere with fairly thick jelly. Velum narrow. Stomach small, about one-twelfth the diameter of the umbrella, attached to subumbrella along arms of perradial cross, leaving small flat triangular pouches between dorsal wall of stomach and subumbrella. Mouth with four short broad lips with slightly folded margins. Four straight radial canals and ring canal narrow. Gonads along four radial canals, linear, with median division; along middle three-quarters of radial canal, not reaching to umbrella margin. Large marginal tentacles hollow, probably up to 24 in number, with elongated swollen bases. Small marginal tentacles solid, probably up to 72 in number, three in each space between two large marginal tentacles. Probably up to 48 open marginal vesicles, two in each space between two large marginal tentacles, each with *c.* 12 to 20, or more, concretions and one black ocellus at base. Diameter *c.* 60 mm. Colour on interradial walls of stomach and large marginal tentacles black.

The specimen has been deposited in the British Museum (Natural History) and has been given the registered number B.M. 1956.4.14.2.

My thanks are due to Captain C. A. Hoodless and the crew of R.V. *Sarsia* who collected these specimens; and to Dr P. L. Kramp for reading through the manuscript.

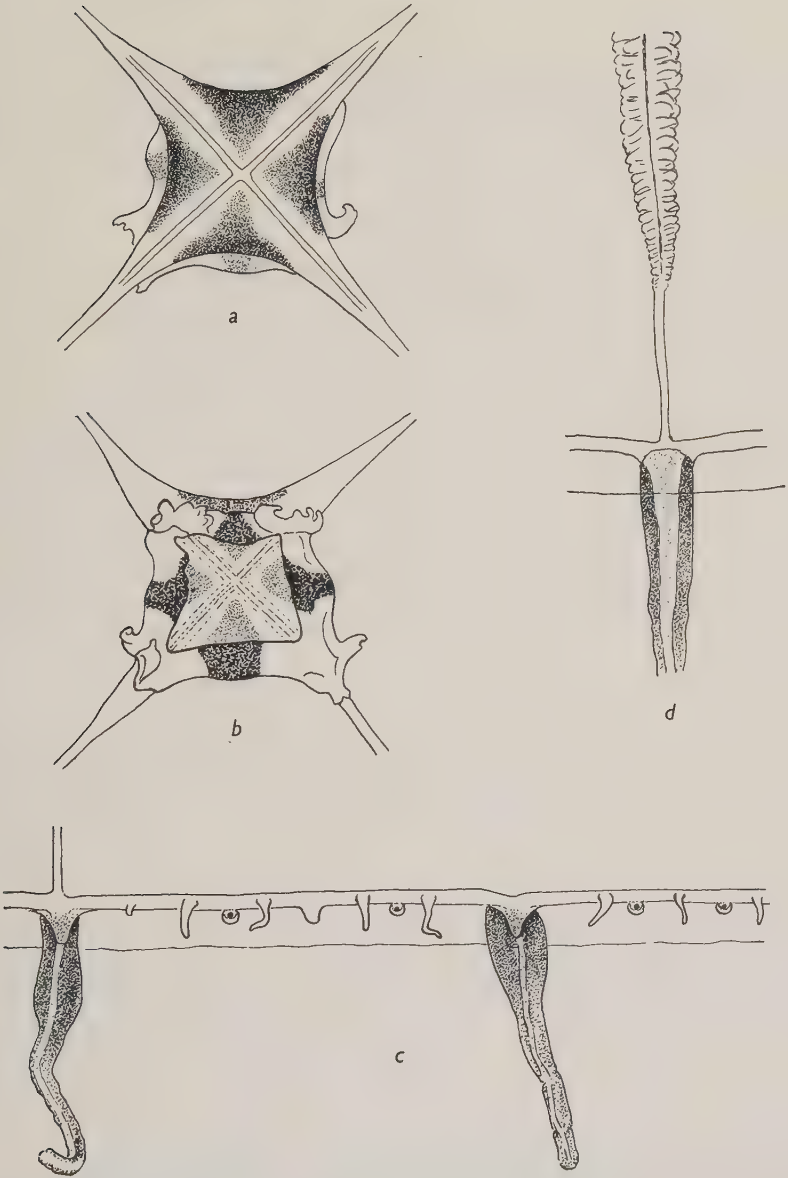

Fig. 3. *Tiaropsidium atlanticum* n.sp. Outline drawings in which dotted areas indicate black pigmentation: *a*, Dorsal view of stomach; *b*, ventral view of stomach; *c*, portion of umbrella margin seen from abaxial side showing two large tentacles, one bulb of large tentacle developing, small tentacles and marginal vesicles; *d*, adaxial view of large tentacle and portion of gonad.

SUMMARY

Single specimens of a new pandeid and of a new mitrocomid medusa were caught in deep water off the mouth of the English Channel in July 1955.

Descriptions are given, and the species have been named *Merga reesi* n.sp. and *Tiaropsidium atlanticum* n.sp. respectively.

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ON THE HYDROID *MERONA CORNUCOPIAE* (NORMAN)

By W. J. Rees
British Museum (Natural History), London

(Text-figs. 1–3)

INTRODUCTION

During a short visit of seven days to the Station Biologique, Roscoff, in June 1955, I was able to make some observations on living colonies of *Merona cornucopiae* (Norman) and on other hydroids. The object of my visit was to see living polyps of *Tricyclusa singularis* which were also found, but unexpectedly *Merona* was found during dredging and provided the opportunity for a much needed re-description from living material.

I am grateful to Professor Georges Teissier for kindly placing a table at my disposal at Roscoff, and also to Dr Claude Levi and Dr Bertil Swedmark for many kindnesses during my stay.

A RE-DESCRIPTION OF *MERONA CORNUCOPIAE* (NORMAN)

There has been no adequate description of *Merona cornucopiae* since Norman (1864) described the species from preserved material from the Shetlands, although Garstang (1892) described some features of a large colony from Plymouth.

Material

Colonies found on *Glycimeris glycimeris* (L.) (Fig. 1) and *Turritella communis* Lamarck, dredged from 15 to 25 m off Callot in the Baie de Morlaix on 23 June 1955, were examined alive, and the following description is based mainly on this material, supplemented by a re-examination of preserved material in the British Museum (Natural History).

The Museum material examined is noted below.

Shetland

On *Dentalium entale* inhabited by *Phascolion*, fertile. B.M. No. 1912.12.21.255. Coll. A. M. Norman (Syntype). This colony is in alcohol; the hydranths have partially disappeared but the perisarcal tubes and the reproductive polyps with their mulberry-like bunches of gonophores remain.

On two shells of *Dentalium entale* with *Phascolion*, sterile, 1867. B.M. No. 1912.12.21.254. Coll. A. M. Norman. There are no polyps and the perisarcal tubes are well covered with adhering sand grains. This colony was probably dead when collected.

Plymouth

On *Aporrhais pes-pelecani* inhabited by *Phascolion strombi*, sterile. B.M. No. 1910.10.1.1. Coll. A. M. Norman. The hydranths are well retracted into the perisarcal tubes which have a height of 3·0–4·0 mm. The tubes have a diameter of about 0·14 mm at the base and expand gradually distally and have a diameter of about 0·35 mm at the rim.

On two living *Dentalium entale*, sterile, Eddystone Grounds, 26 May 1898, B.M. No. 1948.9.8.53. Coll. E. T. Browne. No hydranths or gonophores are present. The taller perisarcal tubes have lengths of 1·98–3·34 mm. The stolons have diameters of 0·06–0·08 mm.

On one living *D. entale*, fertile, Eddystone Grounds, 4 June 1898, B.M. No. 1941.3.20.516. Coll. E. T. Browne. This colony occurs right at the tip of the *Dentalium* shell. The polyps have been killed in an expanded condition and are very life-like. The reproductive polyps have long stalks like the nutritive ones and are further discussed on p. 502. Measurements of this colony are given in Table I.

On a dead *Aporrhais pes-pelecani*, sterile; off Stoke Point, 4 November 1913. B.M. No. 1948.9.8.18. Coll. E. T. Browne. Sterile colony with short stems and polyps moderately extended.

Fragments from the shell of *Glycimeris glycimeris* (L.), sterile, 18 March 1914. B.M. No. 1948.10.1.26. Coll. E. T. Browne. Fragments with few polyps; stems 1·0–3·5 mm high.

Three colonies each on *Dentalium entale*, fertile. B.M. No. 1921.8.31.5 (purchased). Colonies on the apices of the shells; each colony with tall reproductive polyps of the same length as the nutritive polyps.

Eastern Channel, position, 50° 28′ 45″ N., 00° 05′ W.

On *Glycimeris glycimeris* (L.), sterile, 49 m., 24 July 1947. Manihine Collections. A small sterile colony with retracted polyps.

Description

All the colonies seen by me have consisted of 10–40 nutritive polyps, with or without a small number of reproductive polyps, but Garstang (1892) noted a very large colony of 90–100 polyps covering almost the whole upper surface of a shell of *Aporrhais pes-pelecani* inhabited by the sipunculid *Phascolion strombi*. In this colony the hydrorhiza formed a continuous crust, whereas in Norman's colonies and others in the Museum, the stolons sometimes form a crust and sometimes a reticulate network. When the stolons can easily be seen (as on *Dentalium*), they are seen to be covered with smooth, non-annulated, horn-coloured perisarc; they have diameters of 0·06–0·08 mm.

The unbranched polyp stems have a height (to hypostome) of 2·0–5·0 mm when the polyp is expanded. The stems are not usually vertical but slope in the direction of the ciliary currents of the host mollusc; they arise directly from the hydrorhiza with a diameter at base of about 0·14 mm. They expand gradually distally to a diameter of 0·4–0·56 mm. The distal margin is often flared into a trumpet-shape but this feature is more noticeable in young polyps than in old ones which become coated with sand grains or fine mud. When the polyps die down and regenerate, new tubes arise within the mouths of the old ones as noted by Norman, but this feature is not always apparent and caused Garstang to remark: 'The lines of growth encircling the hydrothecae nowhere project so as to assume a ridge-like form, as shown in Norman's figure.'

The large polyps have a length of up to 5 mm when expanded, and have about twenty long filiform tentacles scattered over the anterior half (Figs. 1, 2). Tentacles proximal to the mouth seem to be arranged in an oral whorl of four to seven in number. The proboscis is bluntly rounded and of an intense white colour but posterior to it the digestive endoderm is orange brown to scarlet in colour, and this fades distally to an uniformly pale tint in the non-tentacular portion of the polyp. When brought in from the dredgings the polyps were contracted into their perisarcal tubes with only the tip of the hypostome and the tips of the oral tentacles visible. They did not expand into full length until nearly 12 hours had passed. In the Roscoff colonies the perisarc is of a pale horn colour and rather opaque; it was usually covered with a fine layer of mud particles. The dimensions of the Roscoff and Plymouth colonies are given in Table I.

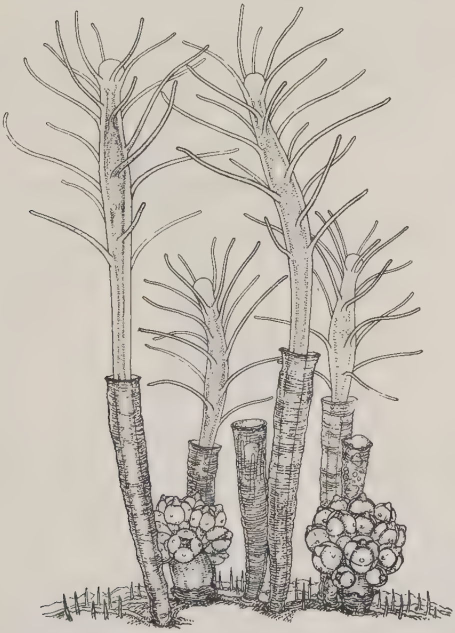

Fig. 1. *Merona cornucopiae* (Norman): a portion of a fertile colony dredged off Callot, Baie de Morlaix, 23 June 1955; drawn from life.

The gonophores in one of the Roscoff colonies were, as Norman stated, borne in the form of mulberry-like masses on very short stalks arising from openings in the stolons. These reproductive polyps have short collars of perisarc at their point of origin on the stolons. It was noticed by Garstang (1892) that 'the blastostyles invariably possess a slender terminal portion, with a slightly dilated apex, which is prolonged beyond the mulberry-like mass of gonophores except when contracted. It is an interesting vestigial structure being homologous with the tentacular portion of a digestive polyp....'

In one of E. T. Browne's well-preserved colonies (B.M. No. 1941.3.20.516) these reproductive polyps had long stalks so that they had a total height of 1·75–3·5 mm, but the cup-like dilations of perisarc at their base had a height of only 0·4–0·6 mm and a maximum diameter of 0·4–0·5 mm (Fig. 2). Each gonophore was pod-shaped, 0·5–0·7 mm long with a diameter of 0·12–0·22 mm. They arise single from the anterior end of the blastostyle and not in any particular order.

Table I. *MERONA CORNUCOPIAE* (Norman)

The Roscoff colony was measured alive and expanded. Browne's colony from Plymouth was killed in an expanded condition after being anaesthetized with cocaine. Measurements in mm.

	Roscoff, 23 June 1955			Plymouth, 4 June 1898			
Total height to hypostome	7·0	6·02	7·35	7·91	6·3	9·59	7·28
Length of polyp	3·78	3·5	4·55	2·66	3·5	5·04	3·08
Diameter of polyp	0·21	0·21	0·21	0·35	0·28	0·28	0·28
Length of tentacle	1·36	1·12	1·33	1·2	1·25	1·3	1·1
Height of stem	3·22	2·52	2·8	5·25	2·8	4·55	4·2
Max. diameter of stem	0·56	0·5	0·52	0·56	0·49	0·56	0·42
Height of reproductive polyp	0·84	0·84	1·2	2·8	1·75	3·5	—
Diameter of gonophore	0·24	0·28	0·32	0·50	0·7	0·6	0·6
Length of gonophore	0·4	0·32	0·35	0·12	0·22	0·22	0·2

By contrast the gonophores of the Roscoff colony were styloid and rather like the eggs of the cuttlefish *Sepia officinalis* in shape; they were round with a teat-like protrusion distally. This protrusion was caused by the tip of the scarlet-coloured spadix. The eggs were pinkish in colour and few in number (two to six). Male gonophores were not seen by me.

Distribution

Although *Merona cornucopiae* is probably not a rare species in our waters, records of its occurrence are few, but these indicate that it may be found all round our coasts except perhaps in the southern North Sea. The species is known from the following localities: Shetland (Norman, 1864), Northumberland (Alder, 1867), 2 miles north-east of Bamborough Castle (Schulze, 1875), the Plymouth area (Garstang, 1892, 1894; Allen, 1899; Crawshay, 1912), the Isle of Man (Moore, 1933, 1937), Blacksod Bay, Ireland (Duerden, 1895) and the Eastern Channel (this paper, p. 500).

Outside British waters the hydroid has been taken at Roscoff (Teissier, 1950), the Faroes (Broch, 1916), the Eastern Kattegat (Kramp, 1935) and in the

Mediterranean (Graeffe, 1884; Motz-Kossowska, 1905). On the North American coast it has been recorded from New England by Verrill (1879) and by Fraser (1936) from Queen Charlotte Islands, British Columbia.

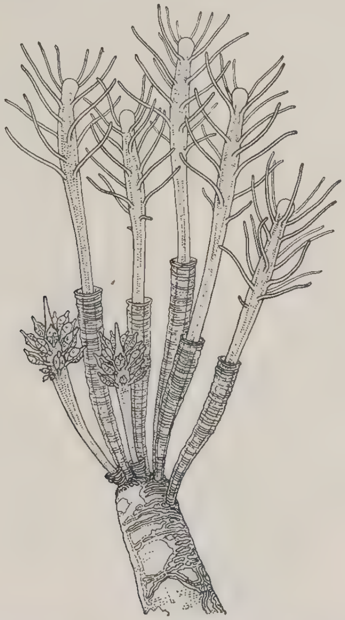

Fig. 2. *Merona cornucopiae* (Norman): fertile colony at the tip of a shell of *Dentalium entale*, Eddystone Grounds, Plymouth, 4 June 1898; E. T. Browne Collection (Brit. Mus. No. 1941.3.20.516). This colony was anaesthetized with cocaine.

Biology

Merona cornucopiae exhibits a preference for the shells of molluscs as a substratum. Where the mollusc is a bivalve like *Glycimeris glycimeris*, the colonies are found at the posterior or postero-ventral margin of the valves, and the stems always lean towards this edge, so that the polyps when expanded project into the ciliary feeding currents of the host. Similarly, the colonies on *Dentalium* are found strategically placed close to the tip of the shell.

In British waters *Merona* has been found on *D. entale* L., *Astarte sulcata* (da Costa), *Glycimeris glycimeris* (L.) and *Venus fasciata* (da Costa), but it has also been found on dead gastropod shells of *Aporrhais*, *Turritella* and *Natica* inhabited by the sipunculid *Phascolion strombi*. Farther afield it has been reported on *Cardium* from the Faroes by Broch (1916) and on an old skeleton of a gorgonian at Banyuls by Motz-Kossowska (1905).

Fertile colonies of *Merona* have been taken at Plymouth in May and June. The species has a bathymetric range of 10–182 m in British waters, but it has been recorded from a depth of 274 m in the Faroes (Broch, 1916).

DISCUSSION

When Norman first described his species *cornucopiae* he placed it in *Tubiclava* Allman 1863, but later decided (1865) that a new genus, *Merona*, must be created for it, because the gonophores were borne in clusters at the tips of short gonoblastidia arising direct from the hydrorhiza. *Merona* was accepted by Allman (1871–72), but not by Hincks (1868), who preferred to retain it in *Tubiclava*. Later authors have been divided as to what genus to use, and the conclusion was reached by Leloup (1930) that *Tubiclava* should be employed. As Leloup did not consider all the facts, the position of the species *cornucopiae* is discussed below.

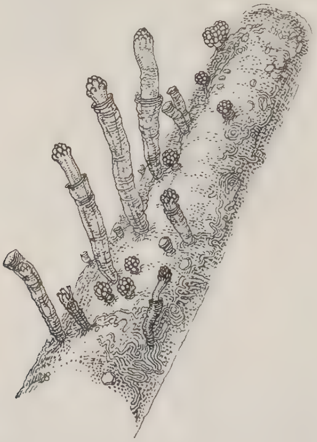

Fig. 3. *Merona cornucopiae* (Norman): a sketch of one of Canon Norman's type colonies on *Dentalium entale* from the Shetlands (Brit. Mus. No. 1912.12.21.255).

The re-description of *M. cornucopiae* shows that the reproductive polyps are sometimes tall and may resemble nutritive polyps in which the tentacles had been resorbed through reproductive exhaustion (comparable to what takes place in species of *Hydractinia*, *Coryne* and *Zanclea*). If this were so, it would narrow the distinction between *Merona* and the more typical *Clava*-like hydroids, but there is no evidence that the reproductive polyp ever possesses tentacles, and it is very significant that the *Merona* reproductive polyp never possesses more than a short collar of perisarc at the base. This last feature indicates that *Merona* possesses two distinct types of polyp and that the reproductive ones are not derived by reduction from the long-stemmed nutritive ones.

Leloup pointed out that *Merona* was distinguished from *Tubiclava* only by '(*a*) les gonophores prennant naissance sur des polypes reduits au blastostyle; (*b*) le perisarque lisse des ses hydranthes et assez large à sa partie pour reçevoir l'hydranthe retracté'. Evidence has already been presented which indicates that the reproductive polyps of *Merona* are special ones, not derived from the ordinary nutritive ones. Leloup based his case on the fact that the hydranths of a Mediterranean species, *Tubiclava pusilla* Motz-Kossowska, became reduced to blastostyles, so narrowing (in his opinion) the gap between the two genera.

Leloup, however, did not consider the status of *T. lucerna* Allman 1863; this, the type species, is a problematic one whose identity is uncertain, for Allman himself later doubted whether he had actually observed fixed gonophores in this species (Allman, 1872, p. 256). *T. lucerna* has a closely adherent perisarc below the hydranth, so that the latter is not retractile as in *Merona*, and more properly belongs to the group of hydroids which have at various times been referred to *Corydendrium*, *Dendroclava* and *Turritopsis*. Allman's second species *Tubiclava fruticosa*, described without knowledge of its gonophores, also belongs to this group (of which only *Turritopsis nutricola* McCrady is known from British waters from its medusa only). I propose to regard *Tubiclava* as a possible synonym of *Turritopsis*, but it should be noted that *Tubiclava pusilla*, on which Leloup based his arguments, does not appear to belong to the *Turritopsis* group, and its status will have to be clarified when it is found again.

Merona Norman may therefore be confidently accepted as a well-constituted genus for *Tubiclava cornucopiae*.

SUMMARY

Merona cornucopiae (Norman) is re-described from living material from the Baie de Morlaix, supplemented by a re-examination of material in the British Museum (Nat. Hist.). *Merona* Norman is demonstrated to be a valid genus while *Tubiclava* Allman (in which *M. cornucopiae* is placed by some authors) is shown to have affinities with the *Turritopsis–Corydendrium* group of clavid hydroids.

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THE ADHESIVE MECHANISMS OF MONOGENETIC TREMATODES: THE ATTACHMENT OF *PLECTANOCOTYLE GURNARDI* (V. BEN. & HESSE) TO THE GILLS OF *TRIGLA*

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(Plate I and Text-figs. 1 and 2)

Little is known of the mechanisms whereby trematode parasites of fish gills attach themselves to their hosts. The adhesive apparatus consists of a posterior set of suckers or clamps supported by skeletal bars (sclerites) whose arrangement varies considerably in the different species. Yet though the pattern of these sclerites forms the main basis for the classification of the 200 or so species of the Diclidophoroidea into its six families, the only attempts to describe the mechanism of attachment of these parasites to their hosts appear to be those of Cerfontaine (1896), who described the attachment of *Diclidophora denticulata* to the gills of *Gadus virens*, and of Sproston (1945*a*) and Llewellyn (1956*a*), both of whom described the attachment of *Kuhnia scombri* to the gills of *Scomber scombrus*. In the present paper the mechanism by which *Plectanocotyle gurnardi* (v. Ben. & Hesse) adheres to its hosts *Trigla cuculus* L., and *T. lineata* Gmelin will be described.

Material was collected at Plymouth during the summers of 1953–55, and investigated by the techniques previously described (Llewellyn, 1956*a*) for *Kuhnia scombri*. In addition, the tendon connecting each extrinsic muscle to its respective clamp was further characterized in histological sections by its positive response to the periodic acid—leucofuchsin routine for the demonstration of polysaccharides (Pearse, 1953), after treatment with ptyalin to remove glycogen from the muscles, and followed by treatment with picric acid to counterstain the muscles and sclerites.

THE ADHESIVE MECHANISM

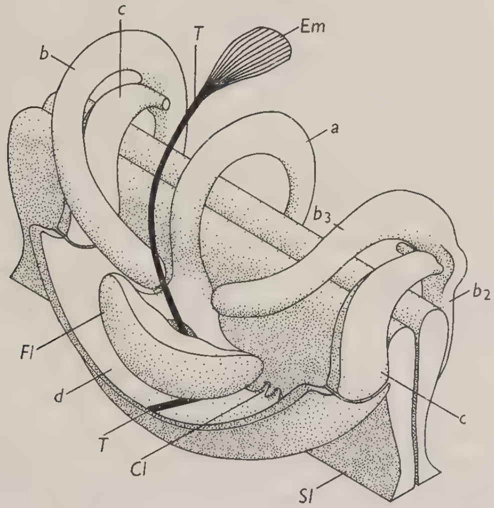
The adhesive apparatus of *Plectanocotyle* consists of three pairs of clamps and a variable number of hooks, all borne ventrally in the posterior region of the body (Pl. I, fig. 1). A pair of anteriorly placed suckers is also present, but these suckers are essentially a component of the feeding mechanism, and will be described elsewhere. The clamps and hooks are all applied to the secondary gill lamellae of one surface of a primary lamella in a manner I have described previously in a comparative account of the adhesive attitudes of gill trematodes (Llewellyn, 1956*b*).

The hooks are borne on a posterior appendage (Pl. I, fig. 1) and function as anchors; such is their efficiency that after separating the clamps from the host tissue during the collection of specimens, it is often impossible to remove the parasite without rupturing the posterior appendage and leaving the hooks still engaged in the secondary gill lamellae. There are invariably two pairs of anchor hooks of which the hooks of the outer pair are about twice as long as those of the inner pair, and sometimes a third much smaller pair of hooks is also present. It is possible that this third pair of hooks may be surviving larval hooks, but an examination of fifteen specimens (histologically fixed and flattened) of *Plectanocotyle gurnardi* revealed no correlation between the size of the specimen and the presence of the third pair of hooks.

Each clamp consists of a postero-ventrally directed pair of hinged opposable jaws with the hinge axis lying approximately transversely across the body, so that one of the jaws lies anteriorly, and the other posteriorly. When the clamp grasps the secondary lamellae of the host, it is the distal end of the posterior jaw that is swung anteriorly about the proximal hinge axis, so that the posterior jaw may be conveniently referred to as the 'movable jaw' and the anterior one as the 'fixed jaw'.

The walls of the clamp consist mainly of fibrous tissue (*Ft* in Pl. I, fig. 4) supported by a framework of skeletal bars or sclerites (*a*, *b*, *c*, *d* and *e*, in Pl. I, figs. 1–8, and Text-fig. 1). Of these sclerites, a single J-shaped one (*a*) lies in the sagittal plane of the clamp. The curved region of the J lies proximally in the clamp, with the short arm extending about half-way into the anterior jaw, and the longer arm extending about three-quarters of the way into the posterior jaw. Sclerite *a* is V- or Y-shaped in transverse section, with the groove facing away from the cavity of the clamp. It is the presence of this groove which gives sclerite *a* the appearance of being hollow when seen in optical section as in Pl. I, fig. 6.

The terminal region of sclerite *a* lying in the posterior jaw is elaborately fashioned to form an important part of the clamping mechanism: the lateral portions of the sclerite, i.e. the arms of the Y, are continued distally and expand to form a lamellate structure that in surface view is approximately semicircular in outline, with the diameter lying proximally and transversely in the posterior jaw of the clamp (Pl. I, figs. 6, 7). The basal region of the sclerite, i.e. the stem of the Y, continues its distal course for a short distance anteriorly to the lamellate expansion, i.e. it forms a short peg running beneath (= nearer to the cavity of the clamp) this lamellate expansion. The lamellate end of *a* does not lie in one plane in the posterior jaw, but curves down over the short peg-like extension of the base of the grooved sclerite, i.e. it curves anteriorly in towards the cavity of the clamp, and then its distal margin is folded posteriorly through 180° so that a transverse groove is formed. In its median region the posterior wall of this groove is greatly thickened, and opposite the centre of the thickened region the groove itself is perforated to permit the passage of a slender tendon (Pl. I, fig. 4) which connects the extrinsic muscles of the clamp with the hinged jaw. From the antero-distal surface of the thickened margin arises a pair of ridges running parallel to the median longitudinal axis of the clamp and which are continuations of the side walls of the perforation (Pl. I, fig. 2). They project towards the cavity of the clamp, and between them enclose a groove which serves as a guide for the tendon. The posterior distal end of sclerite *a* thus constitutes a 'fair-lead' such as I have described (Llewellyn, 1956*a*) in *Kuhnia scombri*.



Text-fig. 1. Stereogram of clamp of *Plectanocotyle gurnardi* grasping secondary gill lamellae of host. *a*, *b*, b_2, b_3, *c*, *d*, sclerites (see text, pp. 508–10); *Cl*, claw; *Em*, extrinsic muscle; *Fl*, fair-lead; *Sl*, secondary gill lamella; *T*, tendon.

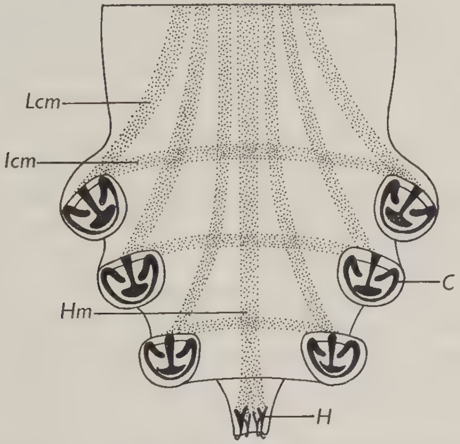
Two systems of sclerites support the peripheral regions of the jaws of the clamp; one of these systems (*c*, *d*) lies wholly within the posterior, movable jaw, while the other (*b*) lies mainly in the anterior, fixed jaw, but extends also into the proximal region of the posterior jaw.

Sclerite *b* passes, on each side of the clamp, along the margin of the anterior jaw (b_1), then curves through the proximal, hinge region of the clamp (b_2), and finally curves again, obliquely medianly into the proximal region of the posterior jaw (b_3) towards its fellow from the opposite side. The distal ends of the two b_1 regions meet each other in the anterior jaw apparently without any actual fusion of sclerite material, while the distal ends of the b_3 regions in the posterior jaw are separated from each other by the median sclerite *a*. Sclerite *b* consists of a flattened bar that in its anterior distal region b_1 presents its narrow edge towards the opposable margin of the posterior jaw; in its proximal region b_2 it bears an outwardly directed shelf-like projection which is grooved on its posterior surface to receive the articulating region of the movable jaw; and in its posterior distal region b_3, sclerite *b* presents a narrow edge distally and a wider surface towards the cavity of the clamp.

The margins of the posterior jaw are supported by three sclerites. In the proximal region of each side is a sclerite *c* consisting of a flattened tapering bar with its wider end lying distally, and with its narrower edges facing anteriorly and posteriorly. The proximal end of *c* bears a peg-like extension directed laterally inwards towards the sagittal plane of the clamp, and which serves as an articulating surface in the hinge joint it makes with the groove of b_2. Sclerite *c* on each side of the movable jaw is joined distally to a median lamellate sclerite *d*. The lamellate nature of this sclerite *d* has usually not been recognized in previous descriptions; the sclerite has been represented by a bar as it appears in Pl. I, figs. 2, 5, but its true nature is shown in Pl. I, fig. 3, where the isolated sclerite has been prepared by digestion with trypsin. The narrow anterior (distal) edge of *d* is opposable to *b* of the anterior jaw, and its efficiency as a clamping device is increased by the presence of a pair of minute anteriorly directed claw-like extensions (Pl. I, figs. 3, 7, 8). The common tendon from the extrinsic muscles of the clamp, after passing through the fair-lead, is attached to the middle of the posterior (proximal) border of *d*.

Lastly, lining the inner walls of the clamp are a number of slender rib-like sclerites *e* (Pl. I, figs. 5, 6, 8), passing, on each side of the mid-line, transversely between sclerites *a* and *b*.

The clamp is closed by the action of a pair of extrinsic muscles, operating by means of a common tendon passing round the fair-lead and attached to the posterior margin of sclerite *d*. On contraction of the muscles, the movable jaw swings anteriorly about its hinge joint with b_2, and secondary gill lamellae are grasped between the opposable edges of *b* and *d*. The course of the extrinsic muscles is illustrated in Text-fig. 2: each clamp is served by two muscles, one of which, the inter-clamp muscle, runs directly across the body to the fellow clamp on the other side, and the other, the longitudinal muscle, turns anteriorly in the body to join with the corresponding muscles from all the other clamps to form a broad longitudinal band of muscle running to the anterior end of the parasite. This pattern of musculature whereby paired clamps are connected to each other by a common inter-clamp muscle obviously increases the efficiency of both the clamps, since each clamp acts as a firm origin for the muscle inserted on the other clamp. It is questionable whether the primary function of the longitudinal muscles is to operate the clamps; they could do so efficiently only when they have a relatively firm origin, otherwise the effects of their contraction would be dissipated into shortening the body rather than bringing about the closing of the clamp. On the other hand, when the anterior end of the parasite is attached to the host during feeding, the efficiency of both the posterior clamping mechanism and the anterior feeding mechanism would be increased. Another possible function of the longitudinal clamp muscle could be the manoeuvring of the anterior end of the body.



Text-fig. 2. The musculature of the adhesive organs of *Plectanocotyle gurnardi*. *C*, clamp; *H*, hook; *Hm*, hook muscle; *Icm*, inter-clamp muscle; *Lcm*, longitudinal clamp muscle.

DISCUSSION

Plectanocotyle gurnardi has been shown to have an adhesive mechanism similar to that of *Kuhnia scombri*. The main differences lie in the number of clamps, there being three pairs in *Plectanocotyle* and four pairs in *Kuhnia*, and in the relative sizes of the hooks in the two trematodes. The large hooks of *Kuhnia* are about twice as long as the proximal-distal axis of one of its clamps, but the large hooks of *Plectanocotyle* are only about half as long as the corresponding length of one of its clamps. Again, while the hooks of *Kuhnia* are borne on the ventral surface of the body proper, those of *Plectanocotyle* are borne on what appears to be a slender posterior appendage, but an assessment of the significance of this is probably best left until the larval development of these parasites has been studied.

Though the clamping mechanisms in *Plectanocotyle* and *Kuhnia* are so similar to each other, there are differences in the detailed arrangement of the supporting sclerites that are of considerable phylogenetic interest. The support given to the median region of the clamp of *Plectanocotyle* by the single sclerite *a* is provided in *Kuhnia* by three quite distinct lamellate sclerites, the two in the anterior and posterior jaws lining the fibrous walls of the clamp, and the one in the proximal region of the clamp lying outside the fibrous wall, i.e. lying between the clamp wall and the remainder of the body. It is probable that a detailed investigation of the development of sclerites and their relationship with other tissues would contribute much to the establishment of the precise homologies of diclidophoroidean sclerites, but here it is suggested tentatively that the rib-like sclerites *e* lining the wall of the clamp of *Plectanocotyle* are represented by the single lamellate sclerite lining the anterior jaw of *Kuhnia*, and that the median sclerite *a* of *Plectanocotyle* corresponds to the other two median sclerites of *Kuhnia*, i.e. those in the posterior jaw and in the proximal region of the clamp. If this be so, and if the fusion of parts to promote greater rigidity, and not fission, be the evolutionary trend, then it would follow that in regard to the median sclerite *a*, the more primitive condition survives in *Kuhnia*. The reverse, however, would obtain in regard to the sclerites of the movable jaw; the more primitive separate nature of the sclerites *c*, *d*, *c* survives in *Plectanocotyle*, but fusion to form a single sclerite has taken place in *Kuhnia*. It is necessary to point out that Sproston (1946, p. 414) ascribed an advantage to the articulated nature of the lateral regions of the 'middle piece' (=movable jaw) of *Plectanocotyle*, in that it enabled the parts to slide over one another and to increase the width of the grasp, 'faintly analogous to the mechanism in the jaws of a snake'. This condition was illustrated by Sproston in her fig. 95*f*, but my observations on *Plectanocotyle* have convinced me that what Sproston has drawn is a clamp skeleton in which the jaws have been forced open and the sclerites disarticulated, possibly by pressure of the cover glass during microscopy.

It is unnecessary to repeat here the criticisms I have made previously (Llewellyn, 1956*a*) of Sproston's (1945*b*) general theory of the evolution of clamping mechanisms in the Diclidophoroidea; it is sufficient to state that in *Plectanocotyle* I have been quite unable to reconcile for example Sproston's description of the 'middle piece' (=movable jaw sclerites) having its 'anterior' (=proximal) border cuticularized and joined with the 'median spring' (=sclerite *a*) by a median cuticularized band, with anything that I have observed in *Plectanocotyle*. As for Sproston's observation that the clamp structure of the Plectanocotylinae is discocotylid in general pattern, it appears to me to be much more like *Kuhnia scombri* than *Discocotyle sagittata*. Furthermore, a preliminary examination of the adhesive mechanisms of the thirteen species of Plymouth diclidophoroideans that I have studied for other purposes (Llewellyn, 1956*b*), indicates that *Plectanocotyle gurnardi*, *Kuhnia scombri* and *Mazocraes alosae* all have very similar mechanisms involving a fair-lead, and all differ substantially from the remainder of these trematodes. If, then, clamp structure is of major phylogenetic significance, *Kuhnia*, *Mazocraes* and *Plectanocotyle* are closely related to each other. Acceptance of this idea would, however, present a new problem: in general, among the Polyopisthocotylea, groups of nearly related parasites are restricted in their distribution to groups of nearly related hosts, e.g. the Diclidophorinae on the Gadidae, and the Cyclocotylinae (= 'Choricotylinae') on the Sparidae; but the three closely related (?) genera *Mazocraes*, *Kuhnia*, and *Plectanocotyle* would then form a group parasitizing such widely divergent host families (Berg, 1947) as the Clupeidae (Clupeiformes), Scombridae (Perciformes, Scombroidei), and Triglidae (Perciformes, Cottoidei).

It is a pleasure to record my thanks to the Director and Staff of the Plymouth Laboratory for collecting facilities, and to Miss Barbara Williams for technical assistance.

SUMMARY

The mechanism by which the monogenetic trematode *Plectanocotyle gurnardi* attaches itself to the gills of various species of *Trigla* has been shown to consist of hooks borne on a posterior appendage, together with three pairs of clamps which, although differing somewhat in structure, act by an extrinsic muscle–tendon–fair-lead–hinged jaw mechanism in exactly the same manner as *Kuhnia scombri* on *Scomber scombrus*. On the basis of clamp structure, *Plectanocotyle* appears to be more nearly related to the Mazocraeidae than to the Discocotylidae in which it is included at present.

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EXPLANATION OF PLATE I

The adhesive mechanism of *Plectanocotyle gurnardi*

Fig. 1. Posterior end of the parasite in ventral view, showing the three pairs of clamps and two pairs of hooks. Due to the flattening of the specimen beneath a cover-glass, the clamps are seen in anterior view.

Fig. 2. The pair of ridges on the anterior surface of the fair-lead which act as a guide for the tendon. (× 40 phase contrast objective used with × 95 substage annulus.)

Fig. 3. The distal median sclerite ('*d*') of the posterior movable jaw, isolated by digestion of the other tissues with trypsin.

Fig. 4. Sagittal section of distal region of clamp, showing the tendon passing around the fair-lead to the movable jaw.

Fig. 5. Clamp in anterior view. (Stained preparation in Canada Balsam.)

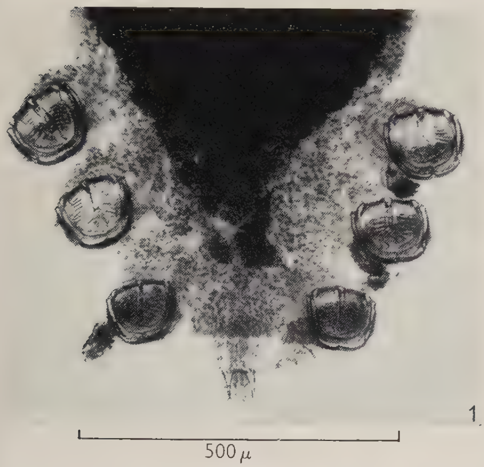
Fig. 6. Clamp in posterior view. (Same clamp as in Fig. 5.)

Fig. 7. Sclerites of a whole clamp remaining after other tissues have been digested with trypsin. The sclerites of the posterior jaw have become disarticulated. Posterior view.

Fig. 8. Another clamp from the same specimen as in Fig. 7, similarly treated. Side view.

Abbreviations

a, *b*, b_1, b_2, b_3, *c*, *d*, *e*, sclerites (see text, pp. 508–10); *Cl*, claw; *Ft*, fibrous tissue; *Fl*, fair-lead; *T*, tendon.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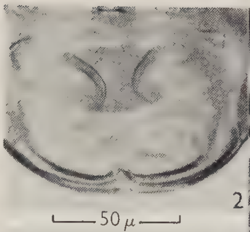
1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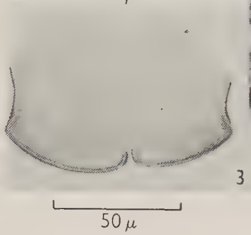
2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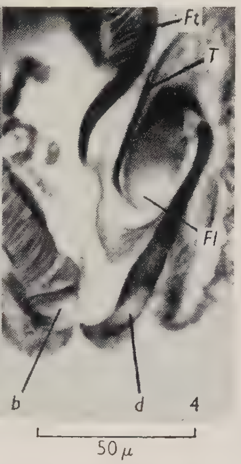
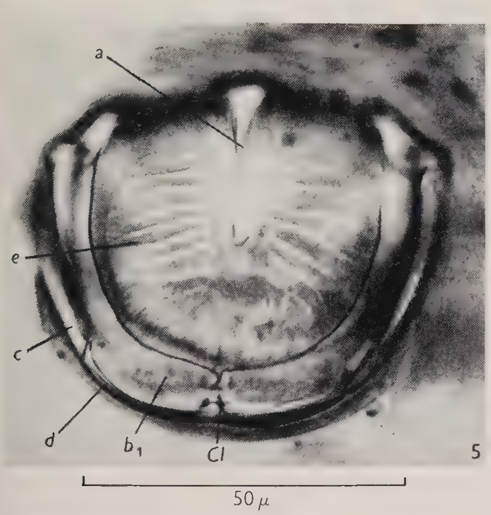
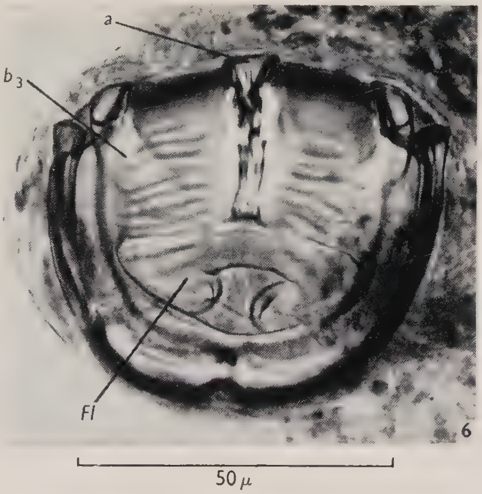
3


Ft
T
Fl
b
d
50 μ

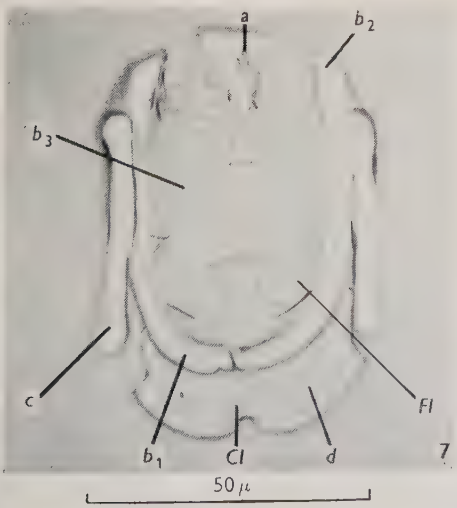
4


a
e
c
d
b_1
Cl
50 μ

5


a
b_3
Fl
50 μ

6


a
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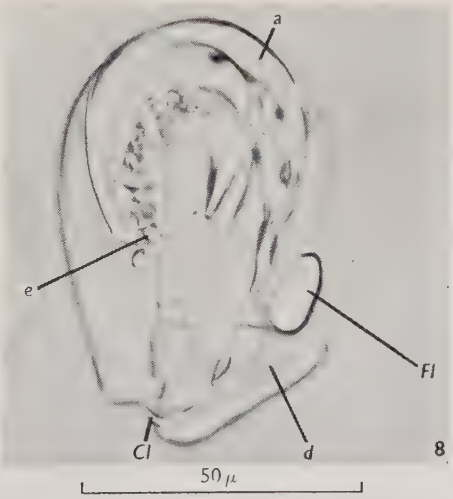
7


a
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Cl
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8

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STUDIES ON THE ENDOCRINOLOGY OF ISOPOD CRUSTACEANS. MOULTING IN *LIGIA OCEANICA* (L.)

By D. B. Carlisle
The Plymouth Laboratory

(Text-fig. 1)

Experiments performed upon decapod crustaceans have produced much information on the hormonal control of moulting in this group. So far as I am aware, however, no one has tried to discover how moulting in other groups of Crustacea is controlled. This note represents the beginning of an attempt to investigate this aspect of moulting in the Isopoda.

OBSERVATIONS ON THE MOULTING PROCESS IN *LIGIA OCEANICA*

It is well known that isopods, unlike most Malacostraca, moult in two portions, shedding the old cuticle of the posterior part of the body sometime before that of the front end. This seems to have been noticed first in *Porcellio* by Schöbl (1879). A detailed account of moulting in some Oniscoidea is given by Herold (1913); Numanoi (1934) describes the process in *Ligia exotica*; Tait (1917) describes it in *L. oceanica*. Briefly, in this species, after a premoult period of 6 or 7 days, a complete separation takes place between the integument of the fourth and fifth thoracic segments; the tergites of the fifth, sixth and seventh segments split longitudinally at the sides (dorso-laterally), and the animal extracts itself from the hinder part of the cuticle by a series of writhing movements. This process takes 10–12 min. The animal walks away using only the anterior walking legs since the posterior ones are not stiff enough to use for at least 12 h. In my experience moulting usually takes place during the hours of daylight. After a variable period (Tait states 4 days at a temperature of 10·5–14.5° C) the anterior thoracic segments split dorso-laterally. Once more the animal extracts itself from the old integument by writhing movements, taking about 30–40 min. I have found that the anterior integument is usually cast in one piece except in underfed animals which have been for sometime in the laboratory. Tait, however, found that this part of the integument was most usually torn into fragments, while the dorsal portions of segments 2, 3 and 4 often remained attached to the animals for some days. This condition I have only seen in animals which moulted in difficult conditions after a period of shortage of food in the laboratory. Indeed, it seems likely that most of Tait's animals were underfed and observed after some lengthy period of captivity, for he states: 'It is a curious fact that in *Ligia*, subsequent to posterior and prior to anterior moult, no increase in the girth of the posterior part of the body can be detected with the eye.... Even when moult is complete *Ligia* is not obviously larger than before'. I find this is only true of starved animals.

The interval between posterior and anterior moult is not nearly so constant as Tait implies. He states that at a temperature of 10·5–14·5° C the interval is 4 days; he makes no mention of any variability. I have found that at a temperature of 15·7–17·4° C the interval is usually 2 or 3 days, but on occasion it may be only 1 day, in either large or small individuals, while periods of 4, 5 or 6 days between the two halves of the moult are not unusual. In five of the animals which I have observed anterior moult has been delayed to the day immediately before the succeeding posterior moult, while in two it has been delayed even beyond this, so that a posterior moult has been followed within 3 days by two anterior moults, one corresponding to the posterior moult of some weeks previously, and one corresponding to the more recent posterior moult.

The average intermoult period at a temperature of 15·7–17·4° C was 47·44 days, corresponding closely with the periods recorded by Nicholls (1931).

THE EFFECT OF EXTRACTS OF *LEANDER* EYESTALKS ON MOULTING RATE IN *LIGIA OCEANICA*

In these experiments about 340 specimens of *Ligia*, freshly collected from Drake's Island, Plymouth Sound, in December 1953, were placed singly in numbered 450 ml. containers in the laboratory each with a piece of *Fucus vesiculosus* for food. Each day the containers were rinsed out with sea water and the weed damped. All moults, deaths and escapes were recorded daily. After a preliminary observation period of 1 week one-third of the animals were injected with an extract of whole eyestalks of female *Leander serratus*. These eyestalks had been excised in August, at a period when they contain a high titre of moult-accelerating hormone, and stored in the refrigerator in absolute acetone. The extract was prepared by draining off the acetone, evaporating the remainder at room temperature, until there was no longer any perceptible aroma of acetone, and grinding the eyestalks with distilled water, acidified to pH 3·8 with hydrochloric acid, in a mortar with washed sand as an abrasive. The extract was filtered and the residue washed with a small measured quantity of acidified water. The total filtrate represented forty eyestalks per millilitre. Each *Ligia* that was injected received 0·05 ml., i.e. two eyestalk equivalents. The injection was made with a tuberculin syringe fitted with a no. 28 needle, inserted in a forward direction between the tergites of the sixth and seventh free thoracic segments into the lateral musculature. No animals died immediately after the injection, but some were found dead 24–48 h later;

these are recorded in Table I. One-third of the animals received no injection; one-third received an injection of acidified distilled water. All animals which had moulted either half of the body during the preliminary observation period were rejected from the experiment.

TABLE I. THE NUMBERS OF DEAD AND OF MOULTS IN EACH GROUP

Days after injection 0	Group 1. Injected eyestalk extract 96 alive		Group 2. Not injected 92 alive		Group 3. Injected distilled water 94 alive	
	Dead	Moults	Dead	Moults	Dead	Moults
1	5	3	1	3	6	2
2	11	13	0	2	8	2
3	0	4	0	2	1	2
4	0	5	0	2	0	3
5	0	2	0	2	0	2
	16	27	1	12	15	11

The dead recorded are those which died without initiating posterior moulting; the moults are the posterior moults which were at least begun, even if the animals died before completion of the moult. The numbers are given only for the 5 days after injection, when there was a significant difference in moult rates; Fig. 1 illustrates the moult rate over a longer period of time.

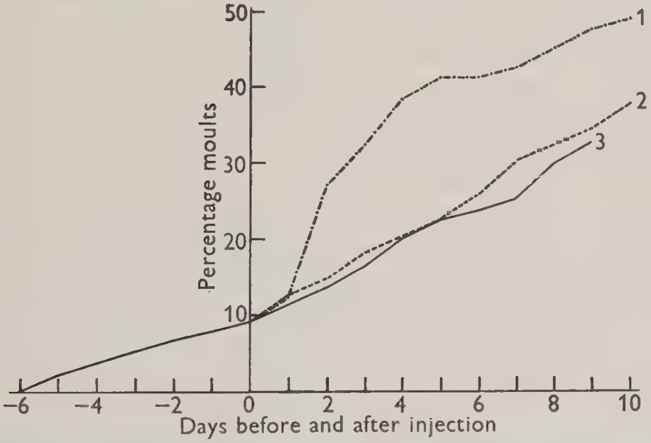

Fig. 1. The percentage moult-rates in the three groups of animals of the first experiment. For explanation see text.

The numbers which died without first moulting and the numbers which initiated the moult of the posterior half of the body are recorded in Table I; some of these latter died without completing the moult; the criterion adopted was simply the initiation of the moult process and the partial withdrawal from the exoskeleton of the posterior half of the body. Parallel probit lines were computed for the moulting rate in the three groups of animals by the method described by Carlisle & Dohrn (1953). The value of Δ_{1-2} was $0\cdot57 \pm 0\cdot23$, which, with 180 degrees of freedom, gave $P < 0{\cdot}01$. The rate of moulting in the group injected with eye-stalk extract was thus significantly greater at the 1% level of probability from that in the uninjected control group and also from that in the control group injected with distilled water. The percentage moulting rate is illustrated graphically in Fig. 1, where it will be seen that after an initial period of accelerated moulting the moult rate in the injected group drops back to normal again, giving a line parallel to that of the controls.

THE EFFECT OF EXTRACTS OF THE OPTIC STALKS OF *LIGIA* UPON MOULTING RATE IN *LIGIA OCEANICA*

I have previously shown (Carlisle, 1953) that the moult-accelerating hormone of Natantia is produced in the eyestalks, within the *X* organ complex. Isopoda, however, are sessile-eyed Crustacea and lack eyestalks. The elements of the *X* organ complex are located instead in the optic stalks of the brain within the head, where also is found the sinus gland. Experiments using extracts of the optic stalks of *Ligia oceanica* were therefore made. They followed the same plan as those of the previous section. Three groups of animals were used, each group consisting of about ninety individuals. Two different extracts were prepared, by dissecting out different portions of the optic stalk. The dissected material was dropped into acetone and the extracts were prepared in the same way as the extract of eyestalks of *Leander*. In the final extract the concentration was once again the equivalent of forty optic stalks per millilitre. Each individual was injected with 0·05 ml. of extract, i.e. two optic stalk equivalents.

The animals of group 4 were injected with an extract prepared from the medulla terminalis of the optic stalk together with the entire *X* organ complex. Those of group 5 received an extract prepared from the sinus glands and the distal portions of the optic stalk. The sixth group received only acidified water. The numerical data and the curves of the moult rate resemble those of the earlier experiment, and will not be given in detail here. The extract of the parts of the *X* organ complex accelerated for a period the moulting rate of group 4 above that of the other two groups ($\Delta_{4-5} = 0{\cdot}49 \pm 0{\cdot}21$, whence $P < 0{\cdot}01$). The moult accelerating principle thus appears, as in the Natantia, to reside in the *X* organ complex and to be absent from the sinus gland.

DISCUSSION

The hormonal control of colour change in Isopoda is similar in many respects to that of Natantia (Kleinholz, 1937; Ståhl, 1938*a*, *b*; Okay, 1945; Suneson, 1947; see also Knowles & Carlisle, 1956). It appears from the experiments reported here that this resemblance extends also to some aspects at least of the hormonal control of moulting. Thus the moult-accelerating principle of the eyestalk of *Leander*, which is known to be active also on the natantian *Lysmata* (Carlisle & Dohrn, 1953) has a similar effect in accelerating the processes of the premoult in *Ligia*. Moreover, extracts of the *X* organ complex of *Ligia* have had the same effect, whereas the sinus-gland extract has had no effect on the moult rate. This agrees well with the conclusion that the moult-accelerating principle in *Lysmata* is present in both the ganglionic and the sensory papilla *X* organs and absent from the sinus gland (Carlisle, 1953). It seems probable then that essentially the same factor is present in the *X* organ complex both of Isopoda and Natantia and is responsible for the control of the course of proecdysis in both.

I have found no evidence that any of my experimental procedures have interfered at all with the interval between the two halves of the moult. It is reasonable to suppose that this somewhat variable interval is under some kind of hormonal control, but if so this is entirely separate from the control exerted by the moult-accelerating principle of the *X* organ complex.

SUMMARY

The moulting process of *Ligia oceanica* is briefly described, and mention is made of the great variability of the period between the two halves of the moult.

The moulting rate of *Ligia* is increased temporarily by a single injection of eyestalk extract of female *Leander*.

The moulting rate is similarly increased by a single injection of extract of the *X* organ complex of *Ligia*, but is unaffected by an extract of the sinus gland and the distal positions of the optic stalk, when this is injected at equivalent concentrations.

The moult accelerating principle of the *X* organ complex of the optic stalk is probably responsible for the control of the course of proecdysis in *Ligia*.

No evidence has been found that eyestalk or optic stalk extracts of the kind used interfere in any way with the length of the interval between the two halves of the moult.

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MECHANICAL PROPERTIES OF *PINNA* ADDUCTOR MUSCLE

By B. C. Abbott and J. Lowy

From the Plymouth Laboratory and Department of Zoology, University of Belfast

(Text-figs. 1–6)

Introduction

It is well known that the smooth adductors of lamellibranch molluscs can hold the shells closed against the tension exerted by the elastic hinge ligament for prolonged periods without visible signs of fatigue. Two opposing hypotheses have been put forward to explain this phenomenon. One postulates that tonic contraction is a tetanic phenomenon and that the economy of lamellibranch smooth muscles is due to their slow speed of relaxation (Ritchie, 1928); whilst the other proposes the existence of a 'catch mechanism' which enables tension to be maintained without expenditure of energy, so that no excitation is needed during tonic contraction (Jordan, 1938).

The mechanical properties of the smooth adductor of *Pinna* have recently been studied in detail by Reichel (1952, 1955) and Bandmann & Reichel (1954), who take the view that though there exists a 'plastic catch-tonus' (based on a mechanism different from that for phasic contraction) continuous excitation is needed to maintain that tonus.

In the experiments described by Bandmann & Reichel, treatment of the smooth posterior muscle of *Pinna* with acetylcholine (ACh) leads to the development of tonus, but it takes about 1 h for the tension to reach maximum. In similar experiments with *Mytilus* muscle we found that maximum tension was reached within 15 sec. This discrepancy, together with Bandmann & Reichel's observation that the *Pinna* muscle was always strongly contracted after isolation—whereas this is not true of *Mytilus* preparations—led us to investigate the *Pinna* adductor.

Methods

The posterior adductor muscle of *P. fragilis* (Pennant, 1777, Edition 4, Vol. 4, p. 97 and pl. 69) was used. It consists of a small white portion facing the hinge and of a larger portion, darker in appearance and containing the longest fibres. Bandmann & Reichel (1954), who worked with *P. nobilis*, do not mention the presence of two macroscopically distinct portions in the posterior adductor. Strips of the muscle containing the longest fibres (dark portion) have been used in the present experiments and these correspond to the preparation described by Bandmann & Reichel.

Histologically, both portions are composed of unstriated fibres and the only distinction between them under the light microscope is that the fibres of the dark portion have a slightly smaller diameter than those which compose the white portion.

Specimens of *P. fragilis* were collected from the region of the Grande Vasière near Concarneau (Brittany). They were kept in running sea water in the Plymouth aquarium and survived satisfactorily for up to 6 months. The posterior adductor muscle was exposed by removing parts of the shells with bone forceps. The visceral ganglia were quickly extirpated. After soaking in aerated sea water for about an hour the muscle relaxed completely under a small load. The length of the muscle with closed shells was from 25 to 30 mm; and relaxed with shells gaping, from 35 to 45 mm. Strips about 4 mm broad and 2 mm thick were prepared from the dark part, tied at both ends and cut. Sea water was used as a bathing medium.

These muscle strips relaxed under a light load to the longest length reached by them in the animal with shells gaping (*reference length*). If the muscle strips were pre-shortened by stimulation below the reference length, allowed to relax, and then stretched, a tension developed which decayed completely within about 1 min. This phenomenon was observed in all smooth and striated muscles which we have investigated (Abbott & Lowy, unpublished) and shows that no resting tension is developed below reference length.

Muscle strips were mounted on a multielectrode stimulating assembly (Hill, 1949) and connected by a light silver chain to an isometric or isotonic lever. Stimulation was by square wave pulses from an electronic stimulator (Attree, 1950). The strength-duration curve for single twitches (threshold responses) was obtained in order to find a suitable pulse width for stimulation. The width generally used was 3 msec, with a voltage of 10 V for maximal stimulation. Contraction was recorded photoelectrically and displayed on a cathode-ray tube.

Records at room temperature were taken of isometric twitches, isometric tetani and of after-loaded isotonic contractions. The stimulus frequency for tetanus was determined from fusion frequency experiments.

The mechanical properties of the resting muscle were investigated by stretches which were applied by lowering a Palmer stand. The resulting tension changes with time were recorded.

Responses to acetylcholine were studied. A final concentration in the bath of 10^{-5} or 10^{-6} g/ml. was adequate to produce maintained tonic contractions.

Some preliminary experiments have been done on the mechanical properties of the small white portion of *Pinna's* posterior adductor: it is much slower than the darker part of the muscle. The latter will from now on be referred to as the fast part of the adductor.

RESULTS

Excitability

Isolated strips of the fast part of *Pinna's* posterior adductor muscle respond to a single stimulus with a twitch. The strength-duration curve for the system is shown in Fig. 1. On the evidence available it is not possible to decide whether this represents excitation of nerve or muscle. Using either multiple-point or one-end stimulation, a maximal isometric twitch can be obtained (Fig. 2). At the recording sensitivity used, the first sign of tension appears at 40 msec (latent period) and the peak twitch tension of 250 g/cm^2 is reached in 200 msec. Relaxation is very slow compared with the rising phase, tension falling to half-value in 2 sec.

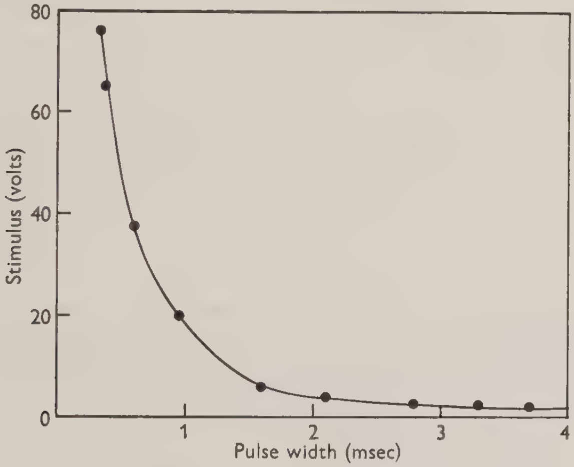

Fig. 1. Strength-duration curve: threshold responses to single stimuli.

Repeated stimuli give responses which summate. There are no signs of facilitation (Fig. 3). The fusion frequency is 7/sec at room temperature and the tetanus tension is about 6 times that developed during a twitch. An isometric tetanus at 7/sec shows a smooth rise of tension which reaches maximum within 4 sec. When stimulation ends the muscle relaxes completely.

Isotonic Shortening

The speed of shortening under afterload conditions varies with load as shown in Fig. 4, the load for zero speed being represented by the isometric tension. The curve is a hyperbola and can be described by Hill's (1938) characteristic relation between force and velocity:

$$(P+a)(v+b)=b(P_0+a),$$

where P is load on the muscle, P_0 is isometric tension, v is speed of shortening, and a and b are constants.

In the muscle illustrated maximum speed of shortening is 8·5 mm/sec or 0·3 length/sec; it is faster than the *Mytilus* adductor (0·1 length/sec). The maximum tension developed by the *Pinna* muscle is only 1·5 kg/cm^2 compared with about 2 kg/cm^2 for many other muscles (Wilkie, 1954), but this is probably due to damage during preparation. The value for a/P_0 is 0·35—well within the range found for a variety of muscle types (Wilkie, 1954).

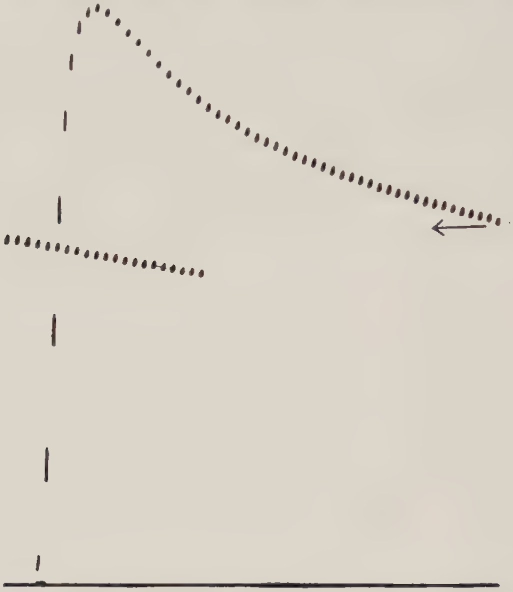

Fig. 2. Record of isometric twitch with multi-point stimulation. Muscle strip, weight 240 mg, length 37 mm, temperature 15° C. Marks of the trace recorded every 20 msec, shock at origin of trace. Time base continuous, tension followed for $1\frac{1}{2}$ sweeps.

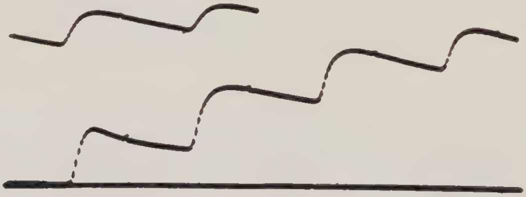

Fig. 3. Repeated twitches, 2/sec. Muscle strip as in Fig. 2.

Length-Tension Relations

The relaxed muscle at reference length shows no resting tension. Tetanic stimulation produces active tension and the value of peak isometric tension decreases on either side of an optimum, at about reference length (Fig. 5).

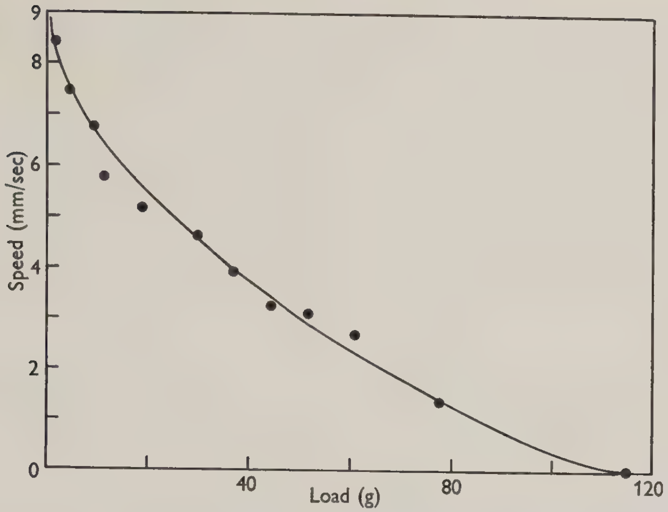

Fig. 4. Force-velocity curve for muscle strip. Weight 250 mg, length 30 mm, temperature 15° C.

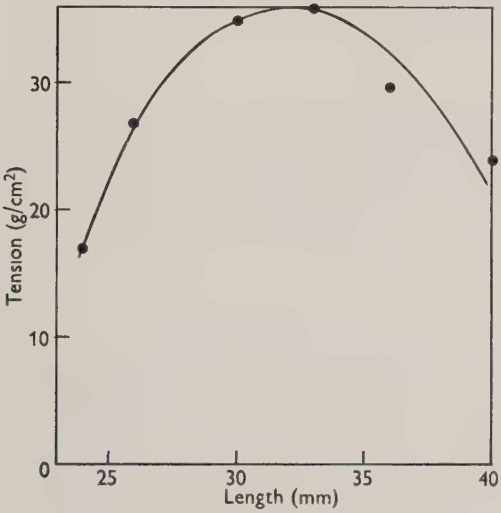

Fig. 5

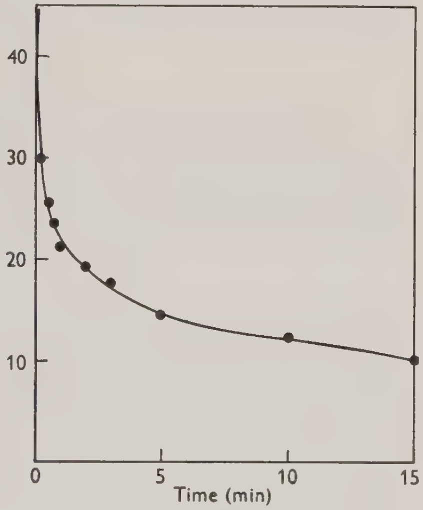

Fig. 6

Fig. 5. Isometric tension-length curve for muscle strip. Temperature 15° C.

Fig. 6. Decay of tension following stretch of resting muscle strip above reference length. Reference length 35 mm, muscle stretched from 35 to 40 mm.

In order to measure active tension at lengths greater than reference length allowance had to be made for resting tension which appears when the muscle is stretched above that length and decays with time as shown in Fig. 6. It was found that at such lengths the resting tension never declines to zero. The procedure adopted was to stimulate the muscle and measure tetanic tension 5 min after it had been extended, by which time the resting tension had fallen to an almost steady value. This gives the right-hand limb of the curve in Fig. 5.

If a long interval was allowed between each stretch so that tension decayed considerably, the muscle could be stretched to about 40–60% above reference length before it showed signs of tearing. The tearing stress is of the order of 6 kg/cm^2.

We find that in *Pinna* muscle, as in *Mytilus* muscle (Abbott & Lowy, 1953), the resting and active length-tension curve can be shifted along the length axis. If the muscle is stretched well beyond its reference length, say by 25%, the tension on it allowed to decay considerably, and the active isometric length-tension curve again determined, its maximum is found to be no longer at the reference length but at a new longer length. However, the value of the peak tension is still the same, i.e. the curve has evidently moved along the length axis. This shift is irreversible and probably due to a 'slip' within the muscle. A similar phenomenon has been observed in vertebrate skeletal muscle (Aubert, Roquet & Van der Elst, 1951).

Tonic Contraction

The *Pinna* muscle responds to application of 10^{-6} ACh by a tonic contraction with up to 75% of maximal isometric tension. The plateau is reached within about 5 sec from the first sign of tension rise. Tension is well maintained for long periods in the presence of ACh but drops gradually to zero after the drug is washed out.

Discussion

Bandmann & Reichel's (1954) hypothesis of the contractile mechanism in lamellibranch smooth muscle may be summarized as follows. Contraction produces shortening and is accompanied by disorientation of fibre, fibrillar and molecular elements within the muscle. This disorientation is long-lasting and associated with an increase in dynamic stiffness due to internal frictional forces. When the muscle is now stretched, these frictional forces resist extension and a state is set up which Bandmann & Reichel define as 'plastic tonus'.

In the animal, excitation causes the adductor to contract against the elastic tension exerted by the hinge ligament. It is assumed that when excitation stops, the frictional forces due to disorientation produced by contraction can 'take over' and resist the pull of the ligament by tension due to 'plastic tonus' (Sperrtonus or catch mechanism). This tension is believed to be the same as that which results from stretch of a resting, shortened, disorientated muscle;

it decays extremely slowly and therefore in the animal very little re-excitation is needed to maintain it.

Evidence for the above hypothesis comes from Bandmann & Reichel's experiments on the mechanical properties of *Pinna* muscle. Length-tension relations of the isolated resting muscle were studied. Stretch produced a tension which decays very slowly. The object of this experiment was to demonstrate the existence of 'plastic tonus'. Next, the decrease in tension as the muscle was allowed to shorten from the stretched condition was investigated. A well-defined length-tension curve could be obtained on which zero tension was reached at the original starting length. But the experiment also showed that 'plastic tonus' can give only a limited amount of tension: if too great a stretch is applied, the muscle 'slips', i.e. if the length-tension curve is again determined, zero tension is reached at a new, longer length. This process, called 'plastic lengthening', is believed to be associated with re-orientation of muscle structures. It is of critical importance to Reichel's hypothesis, for 'plastic tonus' can only disappear by such re-orientation. Thus, in the animal, when the shells gape the muscle is supposed to be plastically lengthened; contraction to result in closure; and 'plastic tonus' to keep the shells closed. That this sequence of events is possible was demonstrated by Bandmann & Reichel in experiments where a plastically lengthened muscle was treated with acetylcholine. Tension was developed and when such an acetylcholine-activated muscle was allowed to shorten in steps and the recovery of tension observed, the length at which no tension was re-developed proved to be the original starting length, i.e. the length before 'slip' occurred.

Taking Bandmann & Reichel's experimental findings as they stand, their hypothesis is both attractive and consistent. But from our experience with other molluscan muscles we were concerned about three observations reported by Bandmann & Reichel. First, that after isolation the *Pinna* muscle was stated to be in a very contracted state, and, even after stretching with a heavy load for a matter of hours, could never be brought back to its body length. Secondly, that at all lengths stretch of the resting muscle resulted in a very long-lasting tension. Thirdly, that the time taken for development of maximum isometric tension following treatment with acetylcholine was about 1 h.

Our experimental results described above indicate that the *Pinna* muscle does in fact behave very much like other molluscan muscles. After suitable dissection it relaxes to maximum body length (reference length); any tension produced by stretch of the resting muscle at lengths below reference length decays completely within a minute; lastly, the time taken to reach maximum isometric tension, whether induced tetanically or by acetylcholine, is only of the order of seconds.

It would thus seem that the muscles used by Bandmann & Reichel were not in good condition. This is substantiated by our observation that if the ganglia are not extirpated rapidly, molluscan smooth muscles often go into a state of 'contracture' and then give much the same responses as those described by Bandmann & Reichel. We have also found that when muscle strips are prepared complete with shell attachments, similar 'contractures' may develop—presumably due to violent excitation whilst cutting the shell close to the muscle strip.

Similar considerations possibly apply to the experiments of Brecht, Utz & Lutz (1955) with *Anodonta* adductors in which the muscles were slow to contract and failed to relax completely after tetanic stimulation. These workers investigated oxygen consumption polarographically and found that the increased rate of metabolism associated with contraction returns to its resting value whilst tension still persists. Brecht and his collaborators conclude from this that a 'freezing' of the contractile elements (catch mechanism) could be responsible for maintenance of tension without increased oxygen consumption. This is contrary to our own findings with *Mytilus* muscle where increased energy expenditure (heat production) accompanies tonic contraction (Abbott & Lowy, 1955).

In our view, the economy of lamellibranch smooth muscle is due to its slow rate of relaxation. The present work on *Pinna* serves to support this hypothesis which has been mainly derived from experiments on *Mytilus* muscle (Lowy, 1953; Abbott & Lowy, 1953, 1955; Hoyle & Lowy, 1956). The fast *Pinna* adductor proved to be much quicker than *Mytilus* muscle; its intrinsic speed is actually comparable to that of some vertebrate striated muscles. On the other hand, as is the case in most other molluscan smooth muscles, the *Pinna* adductor relaxes relatively slowly. Peak twitch tension is reached by 200 msec, but relaxation is complete only after 12 sec. These findings can be considered in terms of the concept of active state (Hill, 1949). The level of active state in a muscle is equated to the tension which the contractile component can exert if it is neither shortening or lengthening. Full activity is developed very soon after stimulation (as shown by quick-stretch experiments). This active state decays with time and at the peak of an isometric twitch, when the contractile and series elastic elements are in equilibrium, the tension exerted represents the level of active state remaining. Since in *Pinna* muscle the tetanus–twitch ratio is about 6, only 1/6 of the full active state remains 200 msec. after the stimulus. Tension, however, decays to zero only after 12 sec. Thus the contractile component returns to a state of complete rest a very long time after the active state has disappeared.

From the above considerations we conclude that tension in the intact *Pinna* adductor cannot be identified with tension due to stretch of isolated resting muscle, i.e. 'plastic tonus' is not a physiological phenomenon. Bandmann & Reichel (1954) and Reichel (1955) agree with the view (Lowy, 1953) that re-excitation is needed to maintain tonic contraction but their concept of the mechanism involved differs basically from our own. Whereas their 'plastic tonus' reflects an internal state of disorientation in resting muscle, we explain the economy of tonic contraction in lamellibranch smooth muscle by the slowness with which the contractile component returns from the active to the resting state.

We wish to thank the officers and crew of *R.V. Sarsia* for their enthusiastic help in locating and collecting *Pinna*. We also wish to acknowledge our gratitude to M. Cailleau of Lesconil, Finisterre who, with his colleagues, guided us to the *Pinna* grounds.

SUMMARY

The mechanical properties of the fast portion of *Pinna's* posterior adductor muscle at rest and during activity have been studied.

The muscle responds to a single shock with a twitch and to repeated shocks with a tetanus. Maximum speed of shortening is about 0·3 length/sec. and peak isometric twitch tension is reached at 200 msec. Relaxation is slow, tension dropping to half in 2 sec and to zero in 12 sec.

Acetylcholine induces a tonic contraction, peak tension being reached in 7 sec. Tonus is only maintained as long as the drug is present.

Contraction in lamellibranch smooth muscle is discussed in the light of Reichel & Bandmann's (1954) hypothesis. On this hypothesis, tonus in the intact muscle is supposed to be maintained by the same mechanism which produces long-lasting tension when resting isolated muscle is stretched ('plastic tonus'). The present experiments show that tension produced by stretch of isolated resting muscle within the limits of body lengths is long-lasting only if the muscle is in poor condition. From this evidence on time relations of muscles in good and poor condition, it is concluded that 'plastic tonus' is not a physiological phenomenon.

The tetanus hypothesis of tonic contraction in lamellibranch smooth muscles is supported, and the economy of these muscles is explained in terms of their slow relaxation rate.

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THE MODES OF ACTION OF TOXIC AGENTS

I. OBSERVATIONS ON THE POISONING OF CERTAIN CRUSTACEANS BY COPPER AND MERCURY

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(Text-figs. 1–5)

Reports of recent attempts to discover how copper and mercury act as poisons to crustaceans are to be found in papers by Clarke (1947), Pyefinch & Mott (1948), Barnes & Stanbury (1948), Hoffmann (1950) and Russell Hunter (1950). Most of the evidence obtained has been interpreted in the light of two general theories (cf. Pyefinch & Mott, 1948). One is that these poisons exert their toxic effects by inactivating vital processes which occur at the animal's surface: the other is that they are absorbed by the animal and act internally by inhibiting metabolic changes. However, conclusive evidence supporting or excluding either possibility has yet to be obtained.

The present account describes some preliminary experiments which have formed part of a series on the effects of toxic agents on crustaceans, and is concerned with testing the importance of penetration in the toxic action of a heavy metal. In these experiments the relative toxicities of copper (as copper sodium citrate) and mercury (as mercuric chloride, ethylmercuric chloride and mercuric iodide) have been tested using (1) larvae of the brine shrimp *Artemia salina* (L.), a species which has been found to be very resistant to heavy metal poisons, and (2) adults of the marine copepod *Acartia clausi* (Giesbrecht) and larvae of the barnacle *Elminius modestus* (Darwin), two crustacean species which, compared with *Artemia*, show little resistance to these poisons. Copper sodium citrate ($NaCuC_6H_5O_7nH_2O$; $Cu = 17{\cdot}5\%$) was used in this investigation because, of the various copper salts tested in experiments with *Artemia*, this was the only one which gave lethal concentrations of copper which were soluble in sea water at pH 8·1. Mercuric chloride was tested because of its widespread use by other workers in this field of research. Ethylmercuric chloride was used because studies of its action as a bactericide and fungicide have led to the view that it possesses a rapid rate of penetration (cf. Sexton, 1953). Finally, mercuric iodide was tested because of the results of further studies carried out in this laboratory (to be published later) concerning the influence of iodine compounds (e.g. sodium iodide and sodium iodoacetate) on the susceptibilities of certain crustaceans to mercury poisoning.

Experiments have also been carried out in which the toxic effects of bipartite mixtures of copper and mercury have been tested, and the results of these studies have provided further information on the modes of action of these poisons which, in some respects, appear to be different.

METHODS

Animals

Artemia salina was reared in sea water. Two days after the larvae had hatched they were removed by filtration on a fine gauze, transferred to a small volume (5–10 ml.) of filtered sea water, and samples (0·01–0·02 ml.) of the thick suspension of animals so formed were then added to the various solutions under test.

Acartia clausi was collected on the day of each experiment with a medium tow-net in Wembury Bay. Separation of these animals from unwanted material was facilitated by their swarming in an area of high light intensity at the surface of the sample, from which they were pipetted first into filtered sea water and then into the toxic solutions.

Elminius modestus larvae were obtained from adults collected from rocks at Tinside in front of the Plymouth Laboratory. The animals were placed in fresh sea water and the larvae which they liberated were collected after 1 h and added to the toxic solutions.

Toxic agents

Solutions of the poisons in sea water were prepared immediately before each experiment and adjusted to pH 8·1. The amounts of mercuric iodide and ethylmercuric chloride which dissolved in sea water were small and limited the range of toxic concentrations which could be studied. However, by shaking suspensions of these compounds in sea water overnight, it was possible to dissolve a concentration of each equivalent to 25 mg Hg^{++}/l.

The concentrations of the mercury and copper compounds used in the present work are expressed throughout in terms of mg Hg^{++} and Cu^{++}/l., a terminology used purely for the sake of convenience in comparing toxicities and not intended to imply that these are the ions to which the compounds give rise in sea water.

Toxicity measurement

Glass tubes (10 by 2 cm), each containing the solution to be tested (5 ml.), and 50–100 animals, were placed on a glass plate marked in squares (0·25 cm^2) and examined under a binocular microscope. Sufficient replicates were used to give a total of 200–300 animals in each of several concentrations of the same poison and in the control solutions. With the three species used it was found that when the animals lost their activity they settled on the bottom of the tube so that the number which lay above each square section of the glass plate could readily be counted. In this way the total number of animals which had lost all signs of movement, including that of their appendages, was estimated at suitable time intervals. These experiments were usually conducted until at least 90% of the test animals had become sufficiently quiescent to be presumed dead. The remaining 10% were then killed (by adding one or two drops of a solution of sodium azide in dil. HCl to the sea-water medium), the total number of animals was estimated, and the percentage of the total which were dead at each time interval was calculated. These percentage values plotted against time gave sigmoid curves, from which the time required for 50% of the test animals to die was calculated. Such '50% death' values were found for each of several concentrations of a given poison. Usually duplicate determinations of the time of 50% death in a particular concentration of any of the poisons used gave values not differing by more than 5% of the mean.

No experiment on *Acartia* and *Elminius* lasted for more than 24 h. Some on *Artemia*, however, were continued for 4 days, during which time animals immersed in toxic solutions and in sea water moulted from the first to the second instar (Heath, 1924). As yet, however, no quantitative examination has been made of the effects of the poisons used on the mechanism of moulting or on the toxicities of these poisons to animals at different stages of development. No food was given to the animals during the toxicity experiments, but both *Acartia* and *Elminius* remained normally active for one day and *Artemia* for at least 5 days in filtered sea water.

EXPERIMENTS AND RESULTS

Comparison of Toxicities

When the time of 50% death was plotted against the concentration the toxicity curves shown in Fig. 1 (*Artemia*), Fig. 2 (*Elminius*) and Fig. 3 (*Acartia*) were obtained. It seemed that the most satisfactory way of estimating the relative toxicities of the poisons to each species was to determine the ratios of the concentrations which caused 50% death in the same time. Because, however, of the marked differences between the toxicities of the poisons used, it was not possible to find a time of 50% death which was common to all four toxicity curves. Accordingly, times of 50% death which were common to the toxicity curves of mercuric chloride, ethylmercuric chloride and mercuric iodide were used to estimate the equitoxic concentrations and hence the relative toxicities of these three mercury compounds; and times of 50% death which were common to the toxicity curves of copper sodium citrate and mercuric chloride were used in the same way in order to calculate the relative toxicities of these two poisons. The toxicities of the four poisons tested with each species, expressed in terms of that of mercuric chloride as a standard, are shown in Table I.

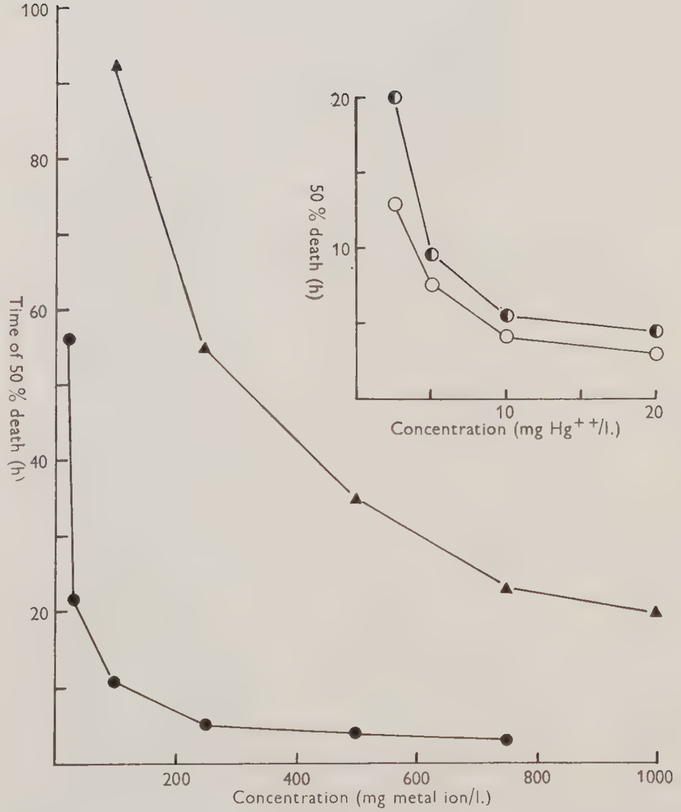

Fig. 1. Survival of larvae of *Artemia salina* in sea water containing copper sodium citrate (▲—▲), mercuric chloride (●—●), ethylmercuric chloride (◐—◐) and mercuric iodide (○—○).

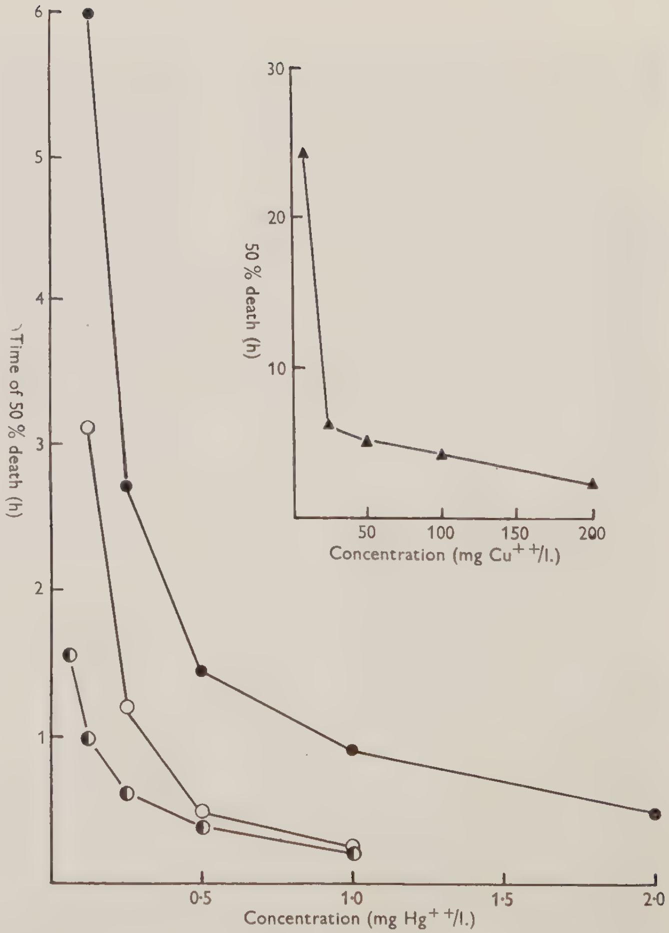

Fig. 2. Survival of larvae of *Elminius modestus* in sea water containing copper sodium citrate (▲—▲), mercuric chloride (●—●), ethylmercuric chloride (◐—◐), and mercuric iodide (○—○).

The results showed that differences between the toxicities of mercuric iodide and ethylmercuric chloride on the one hand and mercuric chloride on the other varied with the resistance to mercury poisoning of the species used as test animal, being minimal with the least resistant *Acartia* (50% death in 2·5 h in 0·05 mg Hg^{++}/l.), slightly greater with *Elminius* (50% death in 2·5 h in 0·30 mg Hg^{++}/l.) and maximal with the very resistant *Artemia* (50% death in 2·5 h in 800 mg Hg^{++}/l.). No such trend was observed, however, in the differences between the toxicities of copper sodium citrate and mercuric chloride: the results simply indicated that copper sodium citrate was much less toxic than mercuric chloride to the three species examined.

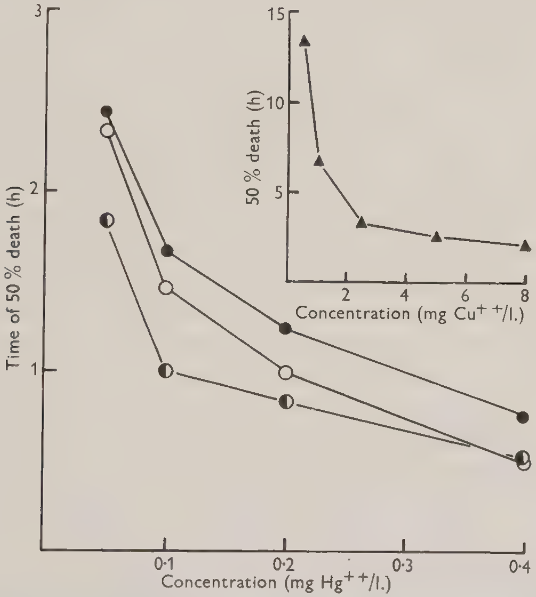

Fig. 3. Survival of *Acartia clausi* in sea water containing copper sodium citrate (▲—▲), mercuric chloride (●—●), ethylmercuric chloride (◐—◐) and mercuric iodide (○—○).

TABLE I. RELATIVE TOXICITIES OF COPPER AND MERCURY POISONS

Compounds in sea water, pH 8·1. Experiments conducted at room temperatures between 21 and 25° C. Toxicity of each poison relative to that of mercury as mercuric chloride, expressed as unity.

	Relative toxicity		
Poison	*Artemia*	*Elminius*	*Acartia*
Mercuric chloride	1·0	1·0	1·0
Ethylmercuric chloride	24	4·4	2·0
Mercuric iodide	31	2·6	1·7
Copper sodium citrate	0·005	0·002	0·01

Studies with Bipartite Mixtures of Poisons

Previous workers have reported that toxic mixtures of copper and mercury show marked supplemental synergisms (more-than-additive toxic effects) when used against crustaceans (Barnes & Stanbury, 1948; Pyefinch & Mott, 1948; Hoffmann, 1950; Russell Hunter, 1950). These observations have led to the view that copper and mercury act on the animals in different ways and that the changes induced by one poison reinforce those caused by the other. To examine this possibility with *Artemia* as the test animal the following procedure was used.

By means of toxicity experiments of the type described earlier certain concentrations of copper sodium citrate (1 g Cu^{++}/l.), mercuric chloride (50 mg Hg^{++}/l.), mercuric iodide (2 mg Hg^{++}/l.), and ethylmercuric chloride (2 mg Hg^{++}/l.) were found which killed 50% of the test animals in approximately the same time. These equitoxic solutions of copper and mercury were then mixed in various quantities to give a series of solutions containing different proportions of the two poisons, and the 50% death time of the test animals in each solution was then estimated in the usual way. If copper and mercury act on the test animal in the same manner then all mixtures of equitoxic solutions of the two poisons should give the same 50% death time. The series of values obtained would then lie on a horizontal straight line (shown as an interrupted line for each mixture in Fig. 4) joining the value for copper sodium citrate only and that found with each of the three respective mercury compounds when used alone. (Actually the solutions of copper sodium citrate and each mercury compound used in these experiments were not exactly equitoxic and the theoretical values therefore lie on lines slightly inclined to the horizontal.) The values found experimentally using mixtures of copper sodium citrate and ethylmercuric chloride are joined in Fig. 4 by a curve which is shown in three sections. The first section is concerned with mixtures containing traces of copper (0–0·05 mg Cu^{++}/l.) and relatively large quantities of mercury (*ca.* 2 mg Hg^{++}/l.) and in this region of the curve it will be seen that the differences between the theoretical and experimental values are insignificant. The second section of the curve includes a value obtained using a mixture of approximately equal quantities of the two poisons (2·0 mg Cu^{++}/l.; 1·9960 mg Hg^{++}/l.), and this again is not significantly different from the theoretical value. The third and largest section of the curve deals with mixtures containing small quantities of mercury and large amounts of copper, and in this region of the curve values found experimentally are consistently lower than the theoretical values. Similar results were obtained when mixtures containing mercuric iodide and copper sodium citrate were studied (central curve of Fig. 4).

The lowest curve in Fig. 4 describes the results of experiments using toxic mixtures of copper sodium citrate and mercuric chloride. The first section of this curve is concerned with mixtures which contained traces of copper (1·0–2·5 mg Cu^{++}/l.) and a large excess of mercury (49·75–49·95 mg Hg^{++}/l.), and it will be seen that, unlike the corresponding sections of the curves described earlier, more-than-additive effects are already manifest. Results depicted in the other section of the curve show that as the quantity of copper in the mixture exceeded that of mercury the differences between theoretical and experimental values became greater until a ratio of 20:1 in favour of copper was reached; thereafter the differences became less marked except for the mixture which contained 975 mg Cu^{++}/l. and 1·25 mg Hg^{++}/l. which again showed a large more-than-additive effect. This latter mixture has been used many times in the course of these studies and has always been found to show a marked supplemental synergism.

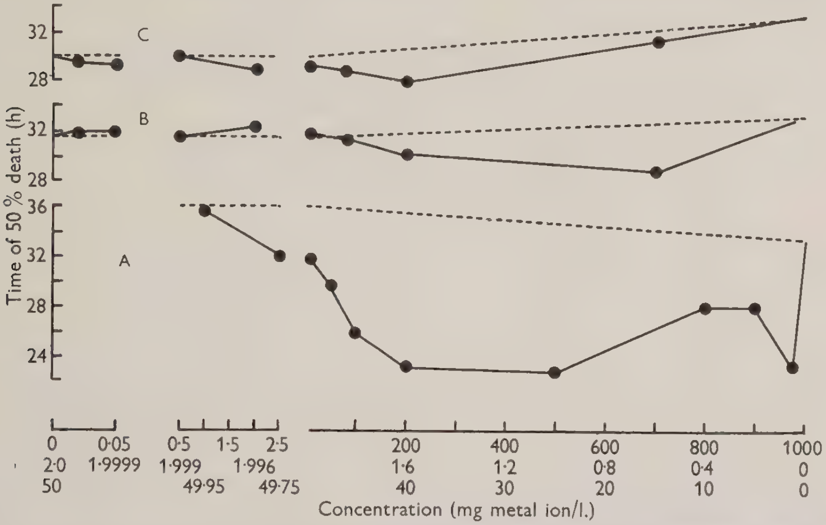

Fig. 4. Effects of bipartite mixtures of equitoxic concentrations of copper sodium citrate and mercuric chloride (*A*), mercuric iodide (*B*), and ethylmercuric chloride (*C*) on the survival of *Artemia* larvae in sea water (●—●, curves obtained experimentally; - - - -, theoretical curves expected if toxic effects of the mixed poisons were exactly additive). Concentrations of the metal ion measured as follows: Cu^{++} as citrate (upper row, 0...1000), Hg^{++} as C_2H_5HgCl or as HgI_2 (middle row, 2·0...0), and Hg^{++} as $HgCl_2$ (lower row, 50...0).

Further experiments of a similar nature were carried out with *Artemia* as the test animal in order to examine the toxic effects of bipartite mixtures of mercuric chloride, mercuric iodide and ethylmercuric chloride. In these experiments, however, theoretical and experimentally determined values were always found to coincide and more-than-additive effects were not observed.

For the purposes of comparison, attempts were made to carry out studies similar to those just described for *Artemia* with the much less resistant species *Acartia*. Because of a shortage of test material, however, experiments with this species were restricted to an examination of the toxic effects shown by mixtures of equitoxic concentrations of copper sodium citrate and mercuric chloride. The results showed that although the mixtures studied gave more-than-additive effects, in nearly every instance the sizes of the effects produced were considerably smaller than those observed when mixtures containing the same proportions of copper and mercury were examined using *Artemia* (see Table II).

TABLE II. COMPARISON OF THE SIZES OF THE SUPPLEMENTAL SYNERGISMS SHOWN BY TOXIC MIXTURES OF COPPER SODIUM CITRATE AND MERCURIC CHLORIDE WHEN TESTED WITH *ARTEMIA* AND *ACARTIA* IN SEA WATER

Synergism expressed as

$$\frac{\text{Time of 50\% death } \textit{theory} - \text{time of 50\% death } \textit{experimental}}{\text{Time of 50\% death } \textit{theory}} \times 100.$$

Ratio of concentrations of heavy metals used in the mixture $Cu^{++}:Hg^{++}$	Synergism	
	Artemia	*Acartia*
1:20	11	11
1:1	20	10
12·5:1	35	8
25:1	32	5
50:1	22	12
100:1	18	16
400:1	26	14

In order to extend this investigation of the causes of the more-than-additive effects produced by toxic mixtures of copper and mercury, experiments were carried out using *Artemia* to examine the possibility that preliminary treatment of the animals with a sublethal dose (i.e. a concentration less than that required to produce measurable lethal effect) of one of the poisons might influence the rate of death of the animals in toxic solutions of the other.

The effect of mercury on Artemia previously treated with copper. A large number of animals were immersed in sea water containing 1 g Cu^{++}/l. as copper sodium citrate. After 1 h in this solution the animals were removed by filtration on a fine gauze, washed several times with plain sea water and then transferred to a series of solutions containing different concentrations of mercuric chloride (250, 125 and 62·5 mg Hg^{++}/l.) mercuric iodide (10, 7·5 and 2·5 mg Hg^{++}/l.) and ethylmercuric chloride (10, 7·5 and 2·5 mg Hg^{++}/l.). For comparison with this copper-treated series, animals were immersed for 1 h in sea water to which no copper had been added and were then transferred to each of a second and similar series of the toxic solutions just described, while samples of these untreated as well as of the copper-treated animals were also placed in plain sea water. The 50% death time of animals present in each solution under test was then estimated in the usual way.

It was found in this experiment that animals which had been immersed in sea water containing 1 g Cu^{++}/l. as copper sodium citrate did not die at a rate significantly faster than that of the untreated animals in either sea water itself or in sea water to which various concentrations of copper had been added. They did, however, die at a much faster rate in each of the sea-water solutions which contained mercury as mercuric chloride and at a slightly faster rate in sea-water solutions of mercuric as mercuric iodide and ethylmercuric chloride (see Table III). Consequently, it seemed that although the amount of poison which was taken up by the animals which were given a preliminary treatment with copper was insufficient to render them less viable in plain sea water or more susceptible to subsequent copper poisoning, it did markedly lower their resistance to the toxic effects of mercury.

TABLE III. THE EFFECT OF A PRELIMINARY TREATMENT OF *ARTEMIA* WITH COPPER ON THE RATES OF DEATH OF ANIMALS SUBSEQUENTLY IMMERSED IN TOXIC SOLUTIONS OF VARIOUS MERCURY COMPOUNDS AND OF COPPER IN SEA WATER

Time of preliminary treatment 1 h. Effect expressed as:

$$\frac{\text{Time of } 50\,\% \text{ death } \textit{untreated} - \text{time of } 50\,\% \text{ death } \textit{treated}}{\text{Time of } 50\,\% \text{ death } \textit{untreated}} \times 100.$$

Poison used to test copper-treated animals	Concn. (mg/l.)	Effect
Hg^{++} as chloride	250	33
	125	48
	62·5	47
Hg^{++} as ethylmercuric chloride	10	21
	7·5	15
	2·5	18
Hg^{++} as iodide	10	18
	7·5	24
	2·5	23
Cu^{++} as citrate	1000	4
	500	3
	250	11

A period of 1 h was used for the preliminary treatment with copper, but

TABLE IV. THE INFLUENCE OF TIME OF PRELIMINARY TREATMENT OF *ARTEMIA* WITH COPPER ON THE RATES OF DEATH OF ANIMALS SUBSEQUENTLY IMMERSED IN TOXIC SOLUTIONS OF MERCURY IN SEA WATER

Concentration of copper = 1 g Cu^{++}/l. as citrate: concentration of mercury = 125 mg Hg^{++}/l. as chloride. Effect expressed as in Table III.

Time of preliminary treatment in copper solution (min)	Effect
5	12
20	34
60	41

later experiments showed that it was possible to induce a considerable influence on the susceptibility of the animals to mercury poisoning by initially exposing them to a copper treatment lasting only 5 min. (see Table IV). On the other hand, prolonging the copper treatment to more than 1 h produced only a slight further increase in the death rate of the animals in the mercury solutions, and in some experiments it was found that after this prolonged treatment the animals died at a rate faster than that of untreated animals in plain sea water.

Effect of copper on Artemia *previously treated with mercury.* These experiments followed a pattern similar to that described previously. The test solutions contained copper sodium citrate (250, 500, 750 and 1000 mg Cu^{++}/l.) and before they were immersed in these solutions, the animals were given a preliminary treatment with either mercuric chloride (250 mg Hg^{++}/l.), ethylmercuric chloride (10 mg Hg^{++}/l.) or mercuric iodide (10 mg Hg^{++}/l.) for 1 h. It was found that animals treated with each mercury compound died at the same rate as that of untreated *Artemia* in sea water and in a series of solutions containing different concentrations of the mercury compound. They died, however, at a rate significantly faster than that of the untreated animals in sea water to which various amounts of copper sodium citrate had been added (see Table V). Apparently, therefore, the quantity of mercury taken up by the animals during their preliminary treatment with either mercuric iodide, mercuric chloride or ethylmercuric chloride was insufficient to render them less viable in sea water or to increase their susceptibility to subsequent mercury poisoning. It did, however, make them far less resistant to copper poisoning.

TABLE V. THE EFFECTS OF PRELIMINARY TREATMENTS OF *ARTEMIA* WITH DIFFERENT MERCURY COMPOUNDS ON THE RATES OF DEATH OF THE ANIMALS IN TOXIC SOLUTIONS OF COPPER IN SEA WATER

Time of preliminary treatment 1 h. Effects expressed as in Table III.

Concn. of Cu^{++} as citrate in solution used to test mercury-treated animals (mg/l.)	Mercury compound used		
	$HgCl_2$ (250 mg Hg^{++}/l.)	HgI_2 (10 mg Hg^{++}/l.)	C_2H_5HgCl (10 mg Hg^{++}/l.)
1000	51	23	26
500	30	25	23
250	42	33	28

In the experiments just described the concentrations of the mercury compounds used to 'sensitize' *Artemia* to copper poisoning had approximately 5 times the potential toxicity of the concentration of copper used in earlier experiments to 'sensitize' the animals to mercury poisoning. Preliminary experiments, however, showed that although concentrations of the mercury compounds of the same potential toxicity as 1 g Cu^{++}/l. 'sensitized' the animals to copper poisoning, the extent to which they so acted varied greatly from experiment to experiment and in some instances the effect was not detected.

Experiments with cysteine and reduced glutathione. It seemed possible that the effect of non-toxic amounts of copper on the susceptibility of *Artemia* to mercury poisoning was sufficiently large to be used as a means of examining the way in which copper acted on this animal. Thus, the possibility was borne in mind that if copper exerted this influence when adsorbed on the surface of the animal, the effect might be abolished if the animal were treated with cysteine, or reduced glutathione, before it was placed in solutions containing mercury. Accordingly, the following experiments were carried out.

TABLE VI. THE INFLUENCE OF CYSTEINE AND REDUCED GLUTATHIONE ON *ARTEMIA* 'SENSITIZED' BY COPPER TO MERCURY POISONING

Effect expressed as in Table III.

Concn. of mercury as mercuric chloride in test solution (mg/l.)	Effect after copper-treated animals washed with		
	Sea water	Glutathione	Cysteine
250	40	27	20
125	49	45	27
62·5	33	25	20

Artemia were immersed for 1 h in sea water containing copper sodium citrate (1 g Cu^{++}/l.). After the animals had been removed from the solution by filtration, approximately equal numbers were added to sea-water solutions of 0·01 M cysteine, and 0·01 M glutathione. Others were added to plain sea water. The animals were suspended in these solutions for 5 min, filtered off, washed several times with sea water and then transferred to solutions of mercuric chloride in sea water equivalent to 250, 125 and 62·5 mg Hg^{++}/l. The rates at which the four sets of animals died in the mercury solutions were compared with those shown by animals which had not been immersed in the copper solution, but simply treated with either cysteine, glutathione or sea water before they were added to sea water containing mercuric chloride. The results of this experiment (see Table VI) showed that animals treated with copper and then washed with sea water died at the same enhanced rate in the mercury solutions. However, copper-treated animals which had subsequently been washed with cysteine or glutathione, died at a rate which, though still enhanced, was usually considerably closer to that shown by animals which had not been given an initial immersion in the copper solution. These findings, therefore, were consistent with the view that some at least of the copper which was instrumental in lowering the resistance of *Artemia* to mercury poisoning might be localized on the surfaces of these animals.

Further, this attached copper was removable with cysteine and glutathione but not with sea water.

Similar experiments were carried out in which animals were immersed for 1 h in sea water containing mercuric chloride (250 mg Hg^{++}/l.), and then washed with either sea water, cysteine or glutathione and transferred to solutions of copper sodium citrate (1000, 750 and 500 mg Cu^{++}/l.) in sea water. The results of this experiment (see Table VII) showed that the mercury-treated animals which had been subsequently washed with cysteine or glutathione died at a rate considerably slower than that of the animals which had been washed in sea water. This finding, therefore, indicated that some, at least, of the mercury responsible for raising the sensitivity of *Artemia* to copper poisoning was attached to the surfaces of these animals, and could be removed with cysteine or glutathione.

TABLE VII. THE INFLUENCE OF CYSTEINE AND REDUCED GLUTATHIONE ON *ARTEMIA* SENSITIZED BY MERCURY TO COPPER POISONING

Effect expressed as in Table III.

Concn. of copper as citrate in test solution (mg/l.)	Effect after mercury-treated animals washed with		
	Sea water	Glutathione	Cysteine
1000	64	42	27
750	61	24	26
500	50	15	25

RESPIRATION EXPERIMENTS

The experiments carried out to investigate the toxic effects of bipartite mixtures of copper sodium citrate and different mercury compounds provided evidence in support of the view that the toxic action of copper reinforces that of mercury, and that when mixtures of copper sodium citrate and mercuric chloride are tested, using *Artemia*, large supplemental synergisms are produced. In order to extend these studies it was considered worth while to compare the effects, if any, of copper (as copper sodium citrate) and mercury (as mercuric chloride) on a fundamental biological activity of the animals, namely their respiration.

Manometric Procedure for Measuring the Respiration Rate of Artemia *Treated with Copper and Mercury*

Oxygen consumption was measured in the conventional Warburg apparatus at a temperature of 25° C and the gas phase used was air. Flasks of approximately 15 ml. capacity equipped with single sidearms were used. Filtered sea water (2 ml.) containing 100–150 mg wet weight of larvae was placed in the main compartment of each flask, samples of filtered sea water (1 ml.) to which no additions had been made or to which an appropriate quantity of copper sodium citrate, sodium citrate, or mercuric chloride had been added were pipetted into the sidearm, and 0·2 ml. 20% KOH together with 3 cm^2 of starch-free filter-paper were placed in the centre well. Equilibration was for 30 min., after which time the rate of oxygen consumption was measured at 30 min. intervals for 2 h. The contents of the sidearms were then tipped into the main compartments of the flasks and measurements of respiration rate were continued for a further 5 h. It was found that the rate of oxygen consumption was independent of the shaking rate over the range 70–100 oscillations/min. (5 cm traverse) and determinations of qO_2 (μl. O_2/mg wet wt./h) were unaffected by the quantities of animals used between 100 and 300 mg wet wt. The pH of the contents of the flasks was measured at the beginning and the end of each experiment and was usually found to have changed from 8·1 to 7·5 during the 7 h experimental period.

The Effects of Copper and Mercury on Respiration and Motility

During the time of these manometric experiments (7 h) the respiration rate of the animals in ordinary sea water, or sea water to which an appropriate quantity of sodium citrate had been added (as a control in the experiments in which copper sodium citrate was used), remained linear, and when the animals were inspected at the end of the experiment they appeared to have suffered no loss of motility and showed no signs of damage. In the course of these studies some twenty determinations of the qO_2 of *Artemia* larvae were made and the values obtained were between 1·01 and 1·15 μl. O_2/mg wet wt./h. The respiration rates of the animals which had been subjected to poisoning by copper and mercury, however, decreased considerably during the experiment and the percentage reduction in oxygen consumption was estimated over each hour subsequent to tipping. To compare these changes in respiration rate with changes in motility the following method was used. Exactly 1 h after tipping took place one of the flasks to which a solution of the poison had been added was detached from its manometer, representative samples (0·2 ml.) of its contents were withdrawn and the numbers of animals which had lost all signs of motility in these samples were determined as percentages of the total numbers present. Other flasks were removed exactly 2, 3, 4 and 5 h after tipping took place and their contents were examined in the same way for percentage loss of motility. By this means percentage reductions in respiration and motility were compared during the same time intervals.

Results of typical experiments are shown in Fig. 5. In these experiments the quantity of copper used was 1 g Cu^{++}/l. and the quantity of mercury was 250 mg Hg^{++}/l., concentrations identical with those employed in the 'sensitizing' studies described earlier. The results showed that whereas copper caused a marked and immediate decrease in respiration, this poison had only a small effect on motility. By contrast, mercury reduced motility faster than it inhibited respiration. A reduction in motility might be expected to precede a fall in respiration on the grounds that animals in a completely motionless state might still possess a small but measurable oxygen consumption. Consequently the action of mercury was considered to be typical of many poisons. The effect of copper on the animals, however, appeared to be one of specific inhibition of respiratory mechanisms, and this finding indicated a difference between the modes of toxic action of the two heavy metals. Similar conclusions have been reached by Russell Hunter (1950) from studies using *Marinogammarus marinus*.

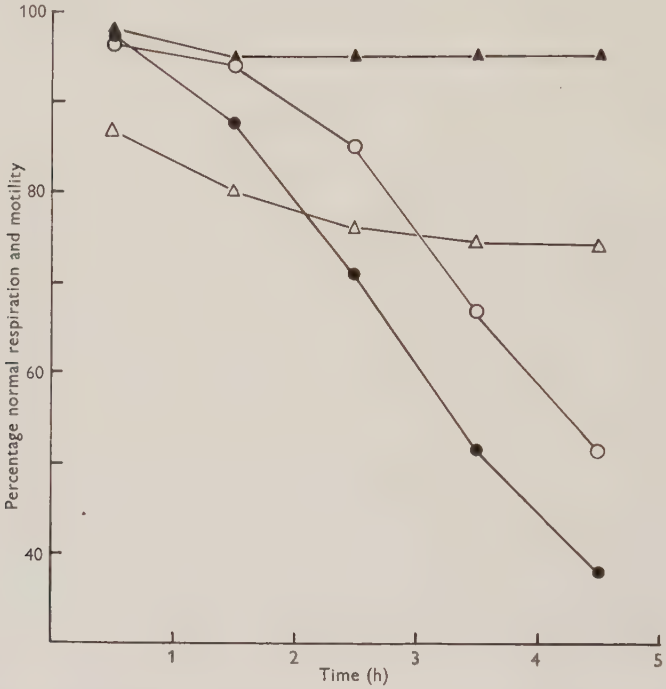

Fig. 5. Effects of copper sodium citrate and mercuric chloride on the motility and respiration rate of *Artemia* larvae in sea water (▲—▲, % decrease of motility in copper sodium citrate; △—△, % decrease of respiration in copper sodium citrate; ●—●, % decrease of motility in mercuric chloride; ○—○, % decrease of respiration in mercuric chloride).

Discussion

The experiments described above were mainly of an exploratory nature and therefore only tentative conclusions may be drawn from their results. Even so the findings reported are worth examining in the light of current theories concerning the mechanisms of action of heavy metal poisons.

At the outset of the present work it was found that *Artemia* is far more resistant than either *Elminius* or *Acartia* to poisoning by mercuric chloride, mercuric iodide, ethylmercuric chloride, and copper sodium citrate. In addition it was found that, whereas mercuric iodide and ethylmercuric chloride are far more toxic than mercuric chloride to *Artemia*, all three mercury compounds are of the same order of toxicity to *Elminius* and to *Acartia*. It appears, therefore, that when the test animal used is one which possesses a considerable resistance to mercury poisoning the differences between the toxicities of the mercury compounds are far greater than those observed when these compounds are tested on an animal which is much more readily poisoned by mercury. These results would be expected if differences between toxicities were closely allied with differences between rates of penetration, for the latter probably play an important part in determining relative toxicities to a highly impermeable animal, but exert a much smaller influence when the test animal used is one which is rapidly penetrated. The results of these toxicity studies therefore are consistent with the view that rates of penetration are, in fact, important factors influencing the toxicities of mercury compounds, and a corollary of this is that these poisons act inside the test animal.

This view assists interpretation of the results of studies made in the present work concerning the toxic effects shown by bipartite mixtures of mercury compounds and copper. Thus it has been found, using *Artemia* as the test animal, that whereas large more-than-additive toxic effects are shown by mixtures of copper sodium citrate and mercuric chloride these effects are much less marked when the mercuric chloride used in the mixtures is replaced by the more toxic iodide or ethylmercuric chloride. A possible explanation of these findings is that the copper used in the mixtures enables the mercury compounds to penetrate the test animal more readily, for it might be expected that this effect of the copper would enhance considerably the toxicity of mercuric chloride but influence to a much smaller extent that of the faster penetrating mercuric iodide or ethylmercuric chloride.

Furthermore, if copper increases the permeability of the test animal to mercury poisons it might be expected that this would have more pronounced effects when mixtures of copper and mercury are tested on a species highly impermeable to mercury than when the test animal used is one which this poison penetrates rapidly. Concerning this aspect of copper poisoning, therefore, interest attaches to further studies made in the present work which have demonstrated that the more-than-additive toxic effects shown by mixtures of copper (as citrate) and mercury (as chloride) when tested on *Artemia* are usually far greater than those observed when similarly proportioned mixtures of the two poisons have been applied to *Acartia*.

Although the results so far discussed support the view that mercury compounds are poisons which act internally, other findings during the present work have suggested that additional toxic effects of mercury as mercuric chloride may be induced at the surfaces of the test animals. Thus it has been found that *Artemia* which have been treated with a sublethal dose of mercuric chloride become more sensitive to poisoning by copper, and that this 'sensitizing' effect can be appreciably reduced if the mercury-treated animals are washed with cysteine or with reduced glutathione. These experiments were similar in principle to those carried out by various workers in the course of studies of enzyme inhibition in which it was observed that the inactivation of enzymes such as urease (Hellerman, Chinard & Deitz, 1943), papain (Hellerman & Perkins, 1934) and yeast carboxylase (Stoppani, Actis, Deferrari & Gonzalez, 1953) by small amounts of copper, mercury and various 'mercaptide-forming' substances derived from the latter (e.g. phenylmercuric chloride) could be partially reversed by thiol compounds such as cysteine. These findings have led to the view that heavy metal poisons inhibit these enzymes by attaching to the surfaces at sulphydryl groups responsible for catalytic activity. The results of analogous experiments carried out with *Artemia* in the present investigation, therefore, lend support to the view that mercury (as chloride) becomes attached to the surfaces of these test animals, possibly by interaction with sulphydryl groups, and that when it is adsorbed at these surface sites it effectively lowers the resistance of *Artemia* to copper poisoning. Evidence consistent with the view that at least part of the toxic action of copper may be explained in a similar way has been obtained from complementary studies in which this poison has been found to 'sensitize' *Artemia* to the toxic effects of mercuric chloride: and in which this 'sensitizing' effect has been observed to diminish after the copper-treated animals have been washed with cysteine and reduced glutathione.

Two further significant observations have been made in the course of these 'sensitizing' experiments using *Artemia*. One is that sublethal doses of mercuric chloride, mercuric iodide, and ethylmercury chloride which have been found to render *Artemia* more susceptible to copper poisoning have been observed to show no marked influence on the susceptibility of the animals to poisoning by the respective mercury compound: and the other is that sublethal doses of copper which have been found to lower markedly the resistance of *Artemia* to mercuric chloride and, to a lesser extent, mercuric iodide and ethylmercuric chloride have no significant effect on the susceptibility of these animals to copper poisoning.

These findings, therefore, together with the results of other experiments discussed earlier in which bipartite mixtures of copper and mercury have been used, provide considerable evidence in support of the view that these two poisons act on *Artemia* in different ways, and evidence of one such difference has been obtained from an investigation of the effects of copper (as citrate) and mercury (as chloride) on the respiration rate of *Artemia* larvae. In these studies it has been found that copper depresses the respiration of the animals by approximately 25% without significantly affecting their motility, whereas mercury reduces their motility at a rate much faster than that at which it inhibits their respiration. As the concentrations of copper and of mercury used in these respiration experiments were equivalent to those used in the 'sensitizing' experiments, there can be little doubt that the animals which were treated with copper in these studies had a reduced respiration when they were later placed in the mercury solutions. It seems possible, therefore, that one of the toxic effects ascribed to copper earlier, namely an ability to increase the permeability of *Artemia* to mercury compounds, may be correlated with its effect as a respiratory depressant.

It is clear that further experimental work is needed before a proper conspectus of heavy-metal poisoning in crustaceans can be formed. In experiments already begun attempts are being made to apply histochemical methods to the problem of determining the sites, either on or in these animals, at which heavy metal poisons exert their toxic effects. Other studies are contemplated in which it is hoped that further information concerning the causes of more-than-additive toxic effects will be gained by testing mixtures of poisons as inhibitors of isolated enzymes and multi-enzyme systems.

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SUMMARY

Studies have been made of the toxicities of copper (as copper sodium citrate) and mercury (as mercuric chloride, mercuric iodide and ethylmercuric chloride) to the three crustaceans *Artemia salina*, *Elminius modestus* and *Acartia clausi*.

Compared with *Artemia* the other two species examined have been found to be much more easily poisoned by each of the four toxic agents tested. Moreover, the differences between the toxicities of mercuric iodide and ethylmercuric chloride on the one hand and mercuric chloride on the other have been found to be far greater with *Artemia* than when *Elminius* and *Acartia* were used as the test animals.

Bipartite mixtures of copper (as citrate) and mercury (as chloride, iodide and ethylmercuric chloride) have been found to show more-than-additive toxic effects (supplemental synergisms) when tested with *Artemia*. Whereas the effects shown by mixtures of copper and mercuric chloride were found to be considerable, those displayed by mixtures of copper and either mercuric iodide or ethylmercuric chloride were, by comparison, very small. In

addition, it has been observed that the more-than-additive effects shown by mixtures of copper and mercury (as chloride) to *Acartia* were significantly smaller than those observed when corresponding mixtures were tested using the more resistant species *Artemia*.

Artemia have been found to be markedly 'sensitized' by pretreatment with a sublethal dose of copper to poisoning by mercuric chloride and, to a lesser extent, to poisoning by mercuric iodide and ethylmercuric chloride. Animals so treated, however, were not rendered more susceptible to copper poisoning. Similarly, it has been found that *Artemia* can be 'sensitized' to copper poisoning by pretreatment with mercuric chloride, mercuric iodide or ethylmercuric chloride in sublethal doses which do not render the animals less resistant to the respective mercury compound. These 'sensitizing' effects induced by copper and mercury (as mercuric chloride) have been found to be partially eliminated by washing the treated animals with solutions of either cysteine or reduced glutathione.

Further experiments with *Artemia* larvae have shown that copper depresses their respiration without significantly affecting their motility. Mercury, however, does not appear to have this effect.

The findings made in this investigation have been discussed in relation to current theories concerning the mechanisms of action of heavy metal poisons, and future studies have been indicated.

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THE DENSITY OF THE EGGS OF *CALANUS FINMARCHICUS*

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(Text-fig. 1)

The vertical distribution of plankton organisms and their eggs depends largely on their surface area and density (Eyden, 1923). Gross & Raymont (1942) observed that the eggs of *Calanus finmarchicus* had a density (1·045–1·049 g/cc) greater than that of sea water (1·0235–1·025 g/cc), and that they sank at the rate of approximately 2·5 cm/min at 13° C. These workers pointed out that since the embryo takes 24 h to complete development, the eggs will sink in still water to a depth of 36 m before hatching and yet the data available, although not extensive, suggests that both eggs and nauplii are found most abundantly nearer the surface than this (Nicholls, 1933; Kraefft, 1910). If these observations are correct, then in addition to density and viscosity, there must be a third factor affecting the distribution of these eggs. Unfortunately, Gross & Raymont did not consider the possible effect of viscosity in their method for determining density, and they themselves commented on the disturbing degree of variation in some of their results. Their measurements of the rate of sinking of the eggs in sea water also showed considerable variation. It seemed worth while, therefore, to measure the density of these eggs using the density gradient technique of Linderstrøm-Lang (1937), a method which enables the individual and precise measurement of the density of small living objects, independent of their shape, i.e. surface area. This method was being used in work on *Psammechinus* eggs at the Marine Station, Millport, and, at the suggestion of Dr A. P. Orr, the following measurements were made on the eggs of *Calanus finmarchicus*.

METHODS

The principles of the gradient-tube method have been fully described elsewhere (Linderstrøm-Lang & Lanz, 1938; Løvtrup, 1950; Linderstrøm-Lang, Jacobson & Johansen, 1938). The medium used was a thorotrast-distilled water mixture made isotonic with sea water by the addition of 0·092 g KCl + 3·46 g NaCl per 100 cc of mixture. (Thorotrast, a stabilized colloidal solution of thorium oxide, has a high density, low viscosity, a negligible osmotic pressure, and is relatively transparent. It has been used for gradients in the Carlsberg Laboratory.) An artificial sea-water medium was used because natural sea water was found to cause precipitation of the thorotrast.

The gradient was about 10 cm in height and covered linearly a density range of 1·048–1·098g/cc. Thus a difference in height of 1 mm in the gradient corresponded with a density difference of 0·0005 g/cc. The density standards were small hollow glass beads (of 1–2 mm diameter) previously made and standardized against droplets of standard potassium chloride solutions in bromobenzene-kerosene gradients. These standards were accurate to ± 0·0005 g/cc and the positions of the eggs in the gradient could easily be measured to 0·1 mm with a cathetometer.

Measurements were made at 22 ± 0·01° C on mixed batches of eggs which had been laid during the night and were approximately 12 h old at the time of the experiments. Two determinations on separate days were made, 20–30 eggs being used on each occasion. The eggs fell as a group in the gradient, and their rate of fall was measured with reference to one egg chosen at random. The small amount of water introduced with the eggs from a braking pipette remained at the top of the gradient tube. The gradient itself was not affected because of the large amount of medium contained in the upper reservoir of the gradient tube. The positions of all the eggs and the standards were measured at suitable intervals for up to 2½ h after entry into the gradient. The diameters of several mixed batches of eggs were measured, with an ocular micrometer, with an accuracy of ± 3μ.

RESULTS

The results of the two experiments are shown in Table 1. Fig. 1 shows a typical set of measurements of the position of an egg throughout the hour following its entry into the gradient. This suggests that the eggs should have reached their definitive positions (i.e. the position at which egg and medium have the same density) within 1 h of entry. This is a slow fall and is due to the small size of the eggs, i.e. high surface-area/volume ratio, giving a high viscosity effect. (In the gradient method viscosity may affect the rate of fall of the material but it does not affect its final position in the gradient.) It is clear from Fig. 1 that the ½ h readings are likely to give density values a little too low. The values for 2 h in Expt. 1 and 1½ h in Expt. 2 may be of doubtful value because the eggs had become opaque, i.e. cytolysed, and may have changed in density. However, this seems unlikely, because if the egg shell is intact internal changes will not affect the egg density; and indeed the opaque eggs were quite steady in the gradient. The progressive decrease in sample size in the second experiment was due to eggs which burst and sank rapidly to the bottom of the gradient. Control eggs remained unchanged for 2 h in the thorotrast-saline mixture at room temperature, while others in the artificial sea water showed signs of deterioration.

Thus from Expt. 1 the density of *Calanus* eggs at 22° C appears to be 1·0735 g/cc with a standard deviation for the population of 0·0005 g/cc. The final value in Expt. 2 supports this, although the standard deviation is much larger. If all the measurements be included, except those of the first half-hour, then the results for the two experiments are:

Expt. 1. Mean density = 1·074 g/cc. Standard deviation = 0·00075.
Expt. 2. Mean density = 1·074 g/cc. Standard deviation = 0·0018.

Since the standards were accurate to ± 0·0005, then the density of *Calanus* eggs may be taken as 1·074 g/cc with a population standard deviation of 0·002 g/cc.

TABLE I. DENSITY OF EGGS OF *CALANUS FINMARCHICUS* AS DETERMINED IN THE DENSITY GRADIENT

Expt.	Time since eggs entered gradient (h)	No. of eggs measured	Mean value of density (g/cc)	Standard deviation	Condition of eggs
1	½	22	1·0725	0·00075	Normal
	1	22	1·0735	0·0005	Normal
	2	22	1·0745	0·0006	Opaque
2	½	28	1·0730	0·00025	Normal
	1	13	1·0750	0·0005	Opaque
	1½	11	1·0750	0·002	Opaque
	2½	8	1·0735	0·00125	Opaque

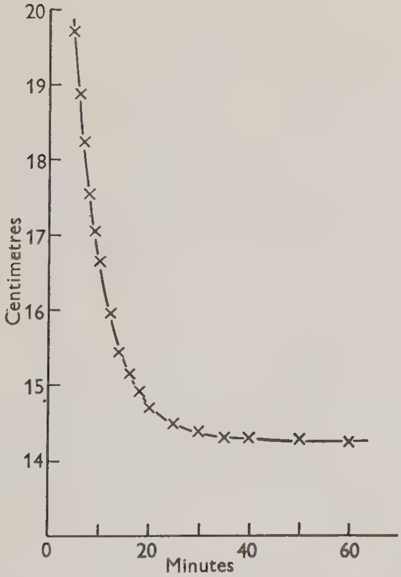

Fig. 1. The fall of an egg of *Calanus finmarchicus* in a thorotrast gradient. The height of the egg above an arbitrary fixed point has been plotted against the corresponding time from the moment of entry of the egg into the gradient.

The results of the diameter measurements are given in Table II. The eggs were, within the accuracy of the measurements, quite spherical. If all the measurements be taken together, the mean diameter for the population becomes 135μ with a standard deviation of 4μ.

TABLE II. DIAMETER OF EGGS OF *CALANUS FINMARCHICUS*

Expt.	No. of eggs measured	Mean value of diameter (μ)	Standard deviation (μ)
1	26	140	3·0
	20	129	3·5
2	25	134	4·0

DISCUSSION

Gross & Raymont (1942) determined the density of *Calanus* eggs by measuring their rates of movement in gum solutions of increasing density, and found that the rate of fall decreased linearly, the egg and the medium having the same density at zero velocity. This, however, only applies if the effect of viscosity is constant or can be neglected, for the rate of movement will be reduced by the increasing viscosity as well as the increasing density of the gum solutions. If the retardation of movement due to viscosity is considerable, as may well be with such small bodies as *Calanus* eggs, then the method becomes very insensitive, giving density values for the solutions in which the eggs just sink or just rise that are too low and too high respectively. In fact there will be a range of solutions in which movements will be very slow and subject to extraneous influences. Indeed, the authors concerned report that different specimens showed marked differences in their rates of sinking—perhaps due to extraneous influences since the measurements were made in glass cells or watch-glasses 2 cm deep and just removed from a refrigerator.

Three values for *Calanus* eggs were determined by Gross & Raymont—a density of 1·045–1·049 g/cc, a rate of fall in sea water of 2·5 cm/min, and a diameter of 190μ. Marshall & Orr (1952) give the mean diameter of *C. finmarchicus* eggs as 140μ. The value of 190μ lies even outside the range which these authors give for *C. helgolandicus* (mean = 172μ). The values for egg density (1·074 g/cc) and diameter (135μ) found in the present work differ considerably from those of Gross & Raymont. Clearly both sets of values cannot be right, unless the eggs of individuals of this species differ markedly in size and constitution, or unless the eggs used in the separate experiments were from a different species.

For further comparison with Gross & Raymont's results it is of interest to calculate, using Stokes's equation, the rate of fall in sea water of an egg with diameter 135μ and density 1·074 g/cc, i.e. with the values determined in the present work. Values for the temperature and salinity of the sea water must be assumed in order to obtain values for density and viscosity. If the sea water has a temperature of 15° C and a salinity of 35‰, then the viscosity will be $12{\cdot}2 \times 10^{-3}$ c.g.s. units (Sverdrup *et al.*, 1942, p. 69) and the density 1·026 g/cc (Knudsen, 1901; Matthews, 1932). Inserting these values in the equation

$$v = \frac{2}{9}\,\frac{a^2 g(d_1 - d_2)}{\eta},$$

where v = rate of fall (cm/sec),
a = radius of egg (cm) = 0·00675 cm,
g = acceleration due to gravity (dynes/cm²) = 981 dynes/cm²,
d_1 = density of egg (g/cc) = 1·074 g/cc,
d_2 = density of sea water = 1·026 g/cc,
η = viscosity of sea water (c.g.s. units) = $12{\cdot}2 \times 10^{-3}$ c.g.s. unit,

we obtain

$$v = 0{\cdot}0393 \text{ cm/sec} = 2{\cdot}36 \text{ cm/min.}$$

Thus the theoretical rate of fall in sea water is in fair agreement with the experimentally determined value of Gross & Raymont, and their comments on the vertical distribution of *Calanus* eggs are still valid. If the previously mentioned reports concerning the abundance of eggs near the surface are accurate, then there must be some factor which materially slows down the rate of sinking of these eggs in the sea. A detailed analysis of their distribution might help in determining the part played by turbulence in this respect.

The results also show that although measurements of the rate of fall of spherical bodies, such as eggs, in sea water can give values for their density, the method is much less sensitive than direct measurements as in the Linderstrøm–Lang gradient method. Furthermore, the possible effect of viscosity must be considered when measuring rates of movement in solutions of different density, and for this reason Gross & Raymont's work on the nauplii and adults of *Calanus* awaits confirmation by methods independent of viscosity.

SUMMARY

The Linderstrøm–Lang density gradient technique has been used to determine the density of the eggs of *Calanus finmarchicus*. The mean density for the population was 1·074 g/cc with a standard deviation of 0·002 g/cc. Measurements of egg diameter gave a mean of 135μ with a standard deviation of 4μ. This would give a rate of fall in sea water (salinity 35‰ and temperature 15° C) of the order of 2·4 cm/min.

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UNDERWATER OBSERVATIONS ON ESCALLOP (*PECTEN MAXIMUS* L.) BEDS

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(Text-figs. 1–5)

INTRODUCTION

In an age-frequency distribution plot of a biological population, it is axiomatic that there should be, in general, a preponderance of young. This expected distribution is not found when sampling some marine animals, notably lobsters and escallops. Many workers, including Priol (1930), Tang (1941), Elmhirst (1945), Baird (1952), Fairbridge (1953), and Mason[1] have noted the absence of young escallops (*Pecten* sp.) in the expected numbers in dredge hauls.

A dredge without teeth, lined with sprat netting, was used at Brixham in an attempt to catch the young escallops, but this dredge filled so quickly with sand, gravel and shell that very few escallops were caught, although those taken did have a generally lower size-range than the escallops caught with a standard toothed dredge. At Castletownbere (Co. Cork) a naturalist's dredge similarly failed to catch many escallops.

The relative scarcity of the young escallops may reasonably be explained in one of two ways:

(1) Settlement may occur in feeder beds, probably inshore, from which, upon reaching a certain size or age, the young escallops migrate back to the parent beds to effect the necessary recruitment.

(2) The young may be present on the beds, but for some reason are not caught.

The first explanation has been fairly widely held, but in some cases, notably in the English Channel beds off Newhaven (Baird, 1952) very long migrations from inshore waters would be necessary. Moreover, recaptures of tagged escallops have never shown any appreciable evidence of migration (Gibson, 1953; Mason[1]). To investigate the second possibility it was decided that direct observations of the escallops on the beds by the use of diving apparatus would be necessary.

The commercial escallop beds in Berehaven Sound, between Bere Island and the mainland, County Cork, were chosen for the initial investigation

[1] J. Mason, Ph.D.Thesis, Liverpool University, 1953, 42 pp.

because escallops were found there in depths ranging from 3 to 14 m (10–46 ft). The Sound is nearly 7 miles long and from one-half to three-quarters of a mile wide. The beds in the Sound had formed the basis for population studies during the preceding 4 years, and a series of tagging experiments had been made (Gibson, 1953).

An exceptionally high proportion of 0-group escallops had been taken during 2 days commercial fishing in December, 1949, at Castletownbere. Of 400 escallops dredged on this occasion 51 were 0-group. This high proportion has not been noted since, and the percentage of 0-group in commercial catches seldom exceeds 2%, but the suggestion remained that the young were present.

It was later found desirable to continue the work at Port Erin, where escallops are found in depths ranging from 17 to 60 m (56–198 ft.), although observations were limited to a maximum of 37 m (120 ft.), and collection and dredging to a maximum of 26 m (85 ft.). Escallops on these beds had been studied by Tang (1941) and Mason.

EQUIPMENT AND METHODS

A Siebe Gorman Aqualung compressed air, self-contained, diving apparatus and a Dunlop underwater swimsuit and swim fins were used.

The standard Irish escallop dredge was used at Castletownbere. This dredge was 1·68 m (5 ft. 6 in.) wide, with a toothed blade. The teeth had an average gap of 8·0 cm, were about 7·0 cm long, and entered the sand to a depth of 1·0–2·0 cm. The dredge bag and belly were made of sisal netting, 6·5 cm between knots, hung square on the dredge frame. The dredge was towed from an 18 ft. boat, without motor, winched by hand up to an anchor.

At Port Erin a standard 1·17 m (4 ft.) dredge completely lined with shrimp netting was used. This dredge had a toothed blade, with a gap of 7·5 cm (3 in.) between the teeth, which projected 5 cm (2 in.) from the bar. The dredge was towed from a 25 ft. motor boat.

The sampling at Castletownbere was done in September 1953. All the sampling was done within a radius of 1 mile (1600 m) at depths ranging from 4 to 14 m (13–46 ft.). Escallops of all sizes were fairly easily seen at all depths in spite of being recessed in the sand and covered with a layer of silt. The overlap of the bottom valve was visible when the valves were closed, and the extruded tentacles were seen when the valves were open. All escallops seen while diving were collected. Consumption of air by a diver is much increased at greater depths, and owing to a shortage of compressed air approximately 70% of the collection by diving was done at the lesser depths; most of the dredging was done at the greater depths because of fouling by annual sea weeds in the shallower water. Thus a possible source of bias was introduced in the sampling; the results of this sampling appear in Fig. 1.

To determine whether the results obtained at Castletownbere resulted from the bias introduced by the distribution of sampling, a further investigation was undertaken at Port Erin, Isle of Man, in July 1955. The diving and dredging were done at random on a bed of escallops off Fleshwick Bay. This bed was approximately half a mile (800 m) square, in depths ranging from 17 to 26 m (56–85 ft.). Although underwater visibility was exceptionally good the escallops were not so easily seen as at Castletownbere. This was probably due to the general reduction of light at the greater depths and to the nature of the bottom, which was of a much rougher and coarser texture than that at Castletownbere. Again all escallops seen were collected and measured. Diving was alternated with dredging, both, as far as possible, being done in the same area. The results of this sampling appear in Figs. 2 and 3.

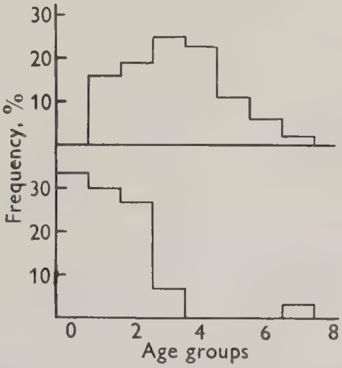

Fig. 1. Age-frequency distribution of collected and dredged samples at Castletownbere. Above: dredged sample, 100 escallops; below: collected sample, 30 escallops.

Movement and Distribution

The escallops normally lie recessed in the bottom in a self-formed depression, the flat valve roughly in the plane of the bottom. Recent swimmers lie unrecessed. The time that elapses after swimming before the recessing is done is not known and is probably very variable. The recessing, which has been observed in action only once, is performed by ejecting water downwards; this is accompanied by a rocking movement. A great deal of the disturbed silt resettles on the upper valve.

The amount of swimming by escallops was very variable but in all cases limited, and occurred most frequently among the smaller ones. In general, not more than 5% of escallops collected by diving swam on the approach of the diver, although on one occasion 10 out of 12 escallops collected swam, but the swimming did not appear to be directional. An escallop could easily be caught whilst swimming. Having swum, and come to rest, no further swimming could be induced. Most frequently a closure of the valves occurred on the initial approach of the diver. Buddenbrock & Möller-Racke (1953) have shown that the reaction of *Pecten* and *Chlamys* to moving objects depends upon the velocity of the object—a slow movement results in a stretching of the tentacles towards the object—while a more rapid movement results in a retraction of the tentacles and closure of the valves. In neither case does the capacity to swim appear to result in an escape reaction and this was confirmed during these observations. Smaller escallops tended to swim more frequently than the larger ones, but even so the very low proportion that were found unrecessed indicated that very little recent swimming had occurred on the grounds examined.

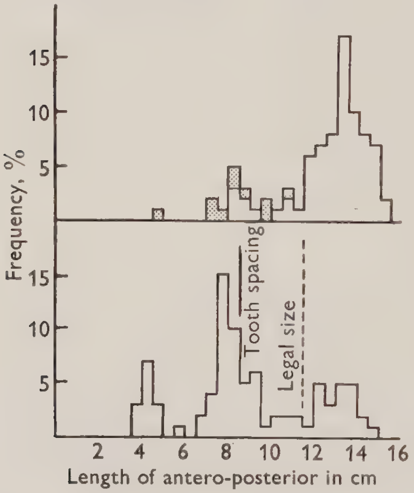

Fig. 2

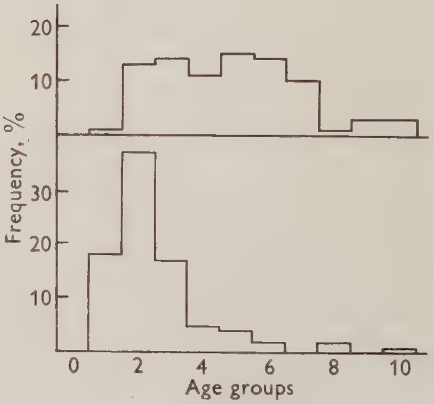

Fig. 3

Fig. 2. Size-frequency distribution of collected and dredged samples at Port Erin. Above: dredged sample, 85 escallops; below: collected sample, 86 escallops.

Fig. 3. Age-frequency distribution of collected and dredged samples at Port Erin. Above: dredged sample, 85 escallops; below: collected sample, 86 escallops.

In an attempt to determine whether any escape reactions occurred among the escallops the dredge was observed in action on the bottom at Castletownbere at a depth of 14 m. In order to see what was happening at the teeth of the dredge it was necessary to look at the bottom immediately in front of the dredge from a height of only half a metre, and this resulted in a high apparent speed over the bottom when the dredge was being towed. Under these conditions it was very difficult to see escallops in the path of the dredge. In the course of the dredge observations only two escallops were positively seen in the path of the dredge, which was moving along in a series of long shallow leaps. Neither of these escallops was taken. On other occasions escallops were taken that were not seen. One 1-group escallop was seen to escape outside the edge of the dredge and was captured by hand. The leaping movement of the dredge must result in a very low dredge efficiency.

The distribution of escallops on the beds was very patchy, estimated densities ranging from one per 2 sq. yd. (1·65 m^2) to one per 100 sq. yd. (84 m^2). There was evidence of localized age grouping which would be heavily masked in dredge sampling. This patchiness appeared to be particularly evident among the lower age groups, though this may have been due to greater ease of identification of the age group of the younger escallops underwater. At Port Erin the 2-group, resulting from a good settlement year, was found almost everywhere but at varying densities.

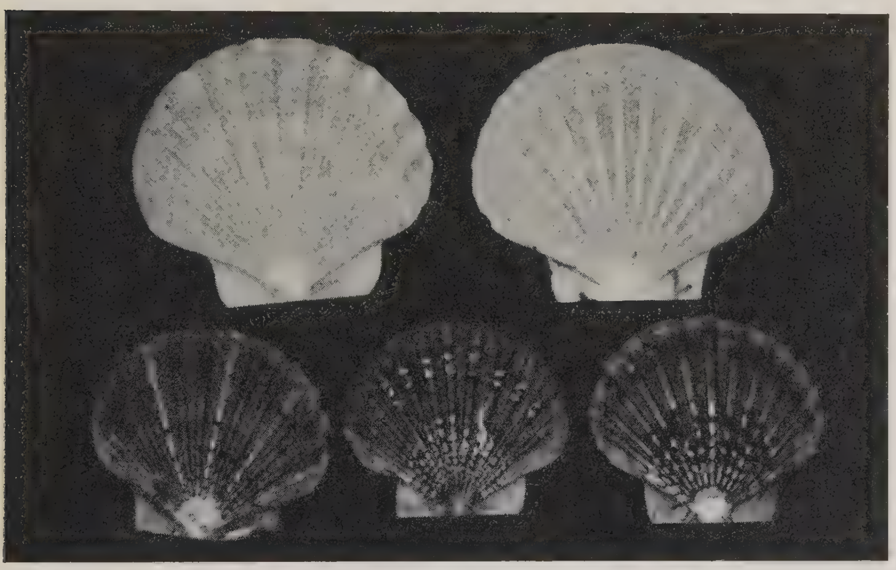

Fig. 4. Contrast in pigmentation of escallops from adjacent areas. The larger escallops are from deeper water and muddy bottom; the smaller from shallow water and gravel bottom.

At Castletownbere a wide range of sizes was found in shallow water on a gravel bottom, but in an area within 200 m (220 yd.) in deeper water on a muddy bottom, the few escallops found were all large. The pigmentation of the shallow water escallops was characteristic and different from that of the adjacent deeper water escallops (Fig. 4). There had obviously been no interchange of populations. A difference in pigmentation was also observed on escallops collected from gravel and muddy bottoms within a mile (1600 m) of each other at Cornwall. The pigmentation in all cases was uniform from the earliest stages of shell growth, indicating that the escallops had been in the same local environment since settlement.

At various intervals from April 1952 until September 1953, 719 escallops have been tagged at Castletownbere, of which 200 (27·8 %) were recaptured by 15 April 1955. Only two have been recaptured in places other than where they were released, and then only within 1 mile (1600 m). Neither can be regarded, therefore, as having made a significant migration. The periods at liberty, ranging from 2 days to 34 months, were adequate for migrations to have taken place, if such migrations are characteristic of escallops. Tagging experiments at Port Erin by Mason have shown a complete absence of migratory movement.

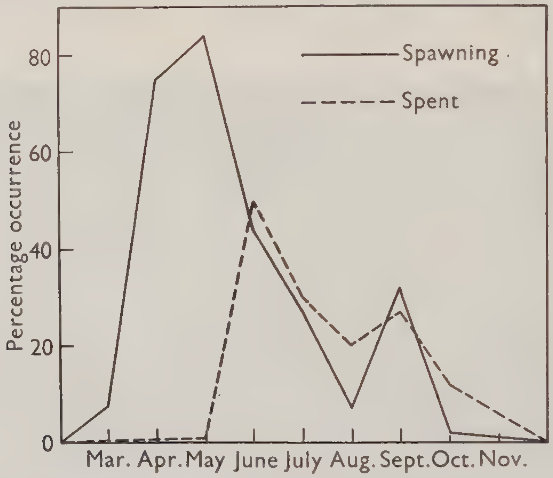

Fig. 5. Spawning and spent escallops in monthly samples.

At Castletownbere 0-group escallops were collected in September. An analysis of the 1953 material has shown that spawning commenced in March, and reached a peak in May, fell away in July and August, and resurged during September (Fig. 5); the early spawned 0-group had attained a size range of 2·9–5·8 cm by September. None were embyssed. At Port Erin, Mason (1953) has shown that the major spawning occurs in the autumn—between 90 and 95 % of escallops on these beds are autumn-spawned—and this will account for the absence of 0-group in the collected sample at Port Erin in July (Fig. 3).

Comparison of Catches by Diving and Dredging

It can be seen (Figs. 1–3) that collection by diving yielded much smaller sizes and lower age-groups than collection by dredging. That this was not due to mesh selection in the bag of the dredge is manifest at Port Erin, where the bag was completely lined with shrimp netting that would retain all escallops. The teeth, which are the only other selective feature of the dredge, must be the effective selection agent.

The shaded squares on the histogram of the dredged sample at Port Erin (Fig. 2) represent escallops caught when the dredge filled with gravel and stopped the boat, i.e. they were caught when the dredge was acting as a complete bottom sampler and the teeth were ineffective as a selecting agent. If the legal size limit (11·5 cm or 4½ in.) is taken as an arbitrary line of size division, the actual distribution as indicated by diving shows 74·4% of the escallops on this bed to be below the legal size, while the dredge retained only 23·5% below this size. The teeth on the dredges, the length and spacing of which are not regulated, are therefore seen to constitute a highly effective saving gear.

Truly representative sampling of escallop beds is difficult. The low densities of escallops makes grab sampling impracticable. Dredges that effectively sample the whole size-range of the population have to have a considerable 'bite' because the escallops are recessed. They thus retain much of the bottom material and are effective for only a short distance. In consequence of the low densities at which escallops occur, such small catches are obtained under these conditions that sampling becomes extremely laborious.

Underwater photography and underwater television have been developed in recent years as methods of observing bottom animals. There are limitations to their use in assessing escallop populations. The area of the bottom observed is small and the escallops would not always be easily seen. The equipment required is relatively expensive. The advantages of the methods lie in their capacity to operate at greater depths and lower temperatures than is possible for a diver using self-contained breathing apparatus.

We would like to acknowledge, with thanks, the co-operation of the Director and staff of the Marine Biological Station at Port Erin in making boats and facilities at the laboratory available.

SUMMARY

Present methods of dredge sampling of escallop beds are unsatisfactory where all age-groups require to be studied. Selectivity by the dredge is continued above the point that might reasonably be expected from mesh size and tooth spacing, the latter being the primary selecting agent. A dredge without teeth and with a fine mesh bag fills with sand and bottom material within a short distance. As the mean density of escallops is low, even on good commercial beds, this results in very small catches.

Escallops of all age-groups are present together on the beds with a tendency towards very localized age grouping, which would not be apparent in dredge sampling. There is no evidence that migration from feeder beds occurs.

There is little apparent escape reaction; the limited reactions seen occurred most often among 0- and 1-group escallops.

Dredge efficiency is low, the Irish dredge used being on the bottom for only a part of the time that it was moving, progression occurring in a series of long shallow leaps.

Conservation of stocks of escallops would be most effective if based on dredge-tooth spacing and size rather than on the size of mesh or rings forming the belly of the dredge.

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THE TIDAL RHYTHM AND ACTION OF THE DIGESTIVE SYSTEM OF THE LAMELLIBRANCH *LASAEA RUBRA*

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(Text-figs. 1–8)

It has been well established that some of the features of the feeding and digestive process in molluscs are rhythmic in character. Notably Hirsch (1915, 1917, 1931) has demonstrated a rhythmic periodicity of secretion in the salivary glands and digestive glands of some carnivorous Gastropoda, and Krijgsman (1925, 1928) has done similar work on the land pulmonate *Helix*. In all of these animals—and in cephalopods, too, where there is a more elaborate nervous control of secretion—discontinuous feeding is the rule. In the other and perhaps larger category of molluscs—those continuously feeding on fine particles—the central mechanism of the gut is the crystalline style, or its forerunner the protostyle. Here the need is, in Yonge's words (1937), 'to the extent to which they depend on extracellular enzymes for digestion, continuous secretion'. In Yonge's view, now classical, the style was regarded as 'an ideal mechanism for the continuous liberation of small quantities of an amylolytic enzyme'. Graham (1939), in his work on style-bearing gastropods, showed that the style is in general confined to animals with a continuous feeding habit, whether by ciliary means or by using the radula to graze on and rasp off fine particles. It is known that style secretion stops and the style is frequently dissolved when animals are removed from the water and cease feeding (see Yonge, 1925).

It has also been suspected in recent years that the digestive gland in continuous feeders displays a well-marked periodicity, with phases of absorption and of ejection of cell contents into the stomach. To some extent the action of the digestive gland in molluscs is still a subject of controversy. Yonge (1926*a*, *b*) made the first thorough study of this gland in a lamellibranch, and held it to be an absorbing organ, ingesting fine solid particles for intracellular digestion. In the opisthobranch gastropods, Fretter (1939) and Graham (1938) elucidated the nature of the digestive gland in detail, and found it to be an organ which, in addition to its ingesting role, had an important accessory role in excretion. Numerous authors, too, have found it to secrete into the stomach. This has always been assumed in carnivores, for example from the work of Hirsch (1915); and Millott's observations (1937) on the nudibranch *Jorunna* showed the formation of what the present writer (1955 *a*) later called 'fragmentation phagocytes'. It was further established that the digestive gland in the primitive pulmonates had a secretory role, which, it was suggested, might be general in microphagous as well as macrophagous gastropods.

In the lamellibranchs, Mansour (1946) made claims for the secretory action of the digestive gland which seemed to go to the length of denying its ingesting role. In this class the problem is simpler, for we are concerned with a gland whose single function appears to be digestive. Several writers on gastropods have suggested an alternation of absorption and secretion in the digestive gland; but the present view, most generally accepted by workers on lamellibranchs, is that such extracellular digestion of proteins and fats as may occur in the stomach is performed by the enzymes from burst or cytolysed phagocytes, and that the 'secretory' role of the digestive gland is confined to the elimination of effete and rejected particles. In a recent investigation of *Lasaea rubra* (Ballantine & Morton, 1956) the authors noted the extreme suitability of that species for a contribution to this problem. *Lasaea* was found to carry out preliminary extracellular digestion of naked or thin-walled flagellates, *Phaeodactylum* ('*Nitzschia*') and diatoms. It furthermore has no gut phagocytes. *Lasaea* lives at a high tidal level, and the periodicity that must thus be imposed on its feeding was thought to offer good scope for an investigation of possible periodicity, both in crystalline style secretion and in the action of the digestive gland. Oldfield (1955) has recently completed a detailed account of the structure of *L. rubra*, including a full description of the gut; and Owen (1955) has published a review of certain aspects of the lamellibranch digestive gland, which is principally concerned with other problems than are discussed here. The writer has benefited greatly by discussion with Mr Owen and from being allowed to read his paper in manuscript.

This work was done while the author was the holder of the University of London table at the Plymouth Laboratory of the Marine Biological Association. His thanks are due to Dr Mary Parke for the generous provision of some of the organisms used in experiments, to Dr G. Y. Kennedy, for much assistance in the investigation of pigments, to Mrs E. Oldfield for a helpful discussion and to Miss D. Ballantine for useful criticisms and suggestions. Finally, the writer has had the great assistance of many discussions with Prof. C. M. Yonge, to whose knowledge of the lamellibranchs and kindly encouragement and criticism of this manuscript he is especially indebted.

THE INGESTION OF FOOD AND THE SECRETION OF THE CRYSTALLINE STYLE

The specimens of *Lasaea rubra* used in this work were gathered from crevices and between barnacle shells near the Hoe (Tinside) bathing pool, at a point that is covered by normal tides for approximately 3 h out of 12. During this time they were able to filter and feed. The relation of the condition of the gut to the tidal cycle was investigated by the daily collection of animals over fortnightly periods and by making routine observations during these periods at the same time each day. An alternative method was to collect animals at hourly intervals during a single day's tides, and also to examine animals experimentally fed after known periods of filtering.

The presence and position of food in the gut was first accurately determined by the fixing, clearing and examining by transparency of sufficiently large samples of animals. This feature was found to show a very constant relation to the position of the tide, so that the immediate past history of a given animal could be fairly reliably ascertained by such an examination of its gut. The nature of the contents of the gut have already been discussed by Ballantine & Morton (1956). Table I sets out the relation between the tidal level and the gut contents of some of the animals considered.

TABLE I. POSITION OF FOOD IN THE DIGESTIVE TRACT OF *LASAEA RUBRA* AT VARIOUS TIMES OF THE TIDAL CYCLE

Sample no.	Hours submerged	Stomach only	Stomach and anterior intestine	Loop of intestine only	Rectum only (faeces in pallial cavity)	Gut empty	No. of specimens examined
1	1	.	16	2	1	5	24
2	2	.	8	4	3	5	20
3	3	3	27	.	.	.	30
4	3½	.	24	1	1	1	27
	Hours exposed						
5	0·3	.	.	11	4	3	18
6	1	.	.	6	7	6	19
7	2	.	.	8	5	.	13
8	8½	.	.	1	1	14	16
9	9	.	.	3	3	16	22

Equally predictable, in relation to the tide, was found to be the size of the crystalline style, and these two features, gut contents and style size, are treated in the next section as the foundation for a third rhythmic feature of the digestive process, the condition of the digestive gland.

From Fig. 1 it becomes clear that during the normal digestive cycle the crystalline style becomes partly dissolved, after the tide has withdrawn, to be rapidly secreted again on the return of the tide. When the stomach is filled with food the style is large and robust, filling the whole length and breadth of the style sac, in which it rotates, with a coiled food string attached to its free end. The style performs perfectly in *Lasaea* the role of a capstan first suggested by Orton (1923, p. 54) and later observed by Yonge (1949) in the Tellinacea, and by Morton (1952) in a number of gastropods. An observation sometimes possible in *Lasaea* strikingly illustrates this role: the style with the coiled string attached could by dissection be slipped from the grip of the style sac, whereupon it was rapidly rotated in the reverse direction to its previous movement by the uncoiling of the tight twist it had imparted to the food string.

The style is reduced to a very small size in the last hours before the return of the tide, and sometimes—though not often—it seems to disappear completely. This was so with the animals in column 12 of Fig. 1. These were at the top of the tidal range during a neap tide, and the visit of the tide must have been too short to re-establish the style. This cannot happen often, and as a rule a style of full dimensions reappears in less than 1 h.

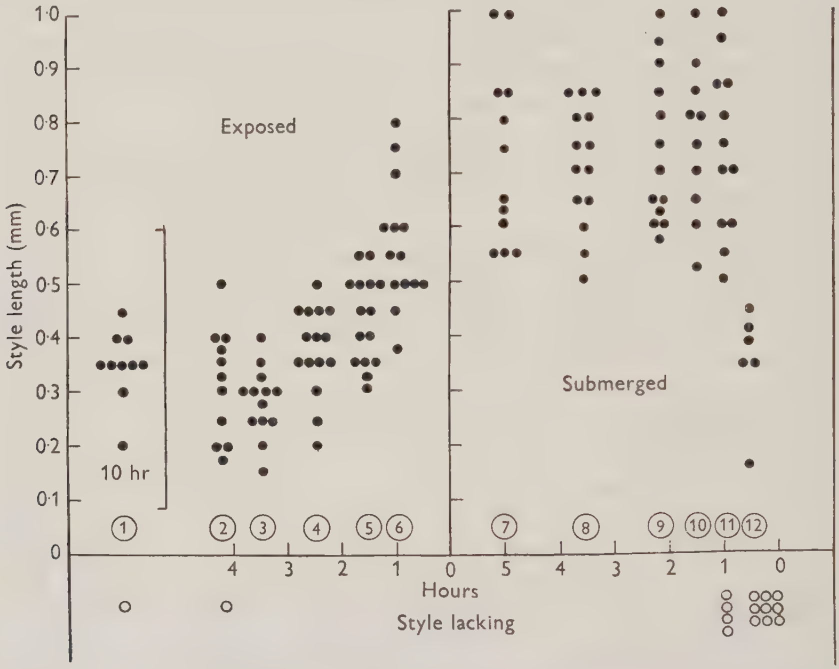

Fig. 1. Diagram showing the relation of the length of the crystalline style to the duration of exposure or submersion of the animal. Clear circles below the zero line represent animals in which the style was found to be absent.

From such observations as were made, the stimulus to the resecretion of the style appears to be the mere resubmersion of the animals. The presence of food in the stomach seems unnecessary, for the style was found to be restored to full size after 1½ h in filtered sea water (Fig. 1, column 10). Here, there may have been some small entry of particles such as debris dislodged from the animal's shell; but even after submergence in cultures of the toxic flagellate *Gymnodinium veneficum*, in which (see Ballantine & Morton, 1956) filtration is prevented, the resecretion of the style was not impeded (Fig. 1, column 11).

SOME EXPERIMENTS ON FEEDING AND DIGESTION

Lasaea rubra is without gut phagocytes at any stage. During this work serial sections have been carefully examined from more than 200 animals, and these amoebocytic cells, which in other lamellibranchs emerge into the lumen to take part in digestion, could never be found. This is possibly a consequence of the small cell size of a minute animal, which may render the phagocytic mode of ingesting particles unsuitable. In this feature *Lasaea* is almost certainly exceptional among the eulamellibranchs. There is as yet no reason to suppose that other lamellibranchs of the Erycinacea may share in this peculiarity; as well as being of small size, *Lasaea* has further specializations peculiar to itself in this group, in particular its extremely high upper tidal limit and the periodicity thus imposed upon its feeding and digestion.

Extracellular digestion of carbohydrates has long been known to take place by the action of the enzymes of the crystalline style. In *Lasaea rubra* there is evidence, from two sources, of extracellular digestion of other substances as well. First, Ballantine & Morton (1956) have already reported in detail the preliminary digestion of *Phaeodactylum* ('*Nitzschia*'), the flagellate *Isochrysis*, and—more slowly—the diatom *Thalassiosira*. All these organisms were digested extracellularly within the lumen of the stomach. With *Phaeodactylum*, digestion could be quantitatively estimated at intervals over a 2 h period by direct counting of organisms in the stomach. The thin cell wall appears to require no cellulase to break it down, and the cell contents, consisting in large part of protein and lipoid reserves, were completely digested out. The cell wall was left as an empty but recognizable 'ghost', sometimes containing traces of the complex carbohydrate leucosin, which appeared to resist digestion longer. The cellulose-armoured dinoflagellate *Peridinium trochoideum* was also fed to *Lasaea*, but was not digested at all.

Evidence of extracellular digestion was also gained from feeding experiments with dogfish erythrocytes. This food, like flagellates, could easily be fed in filterable suspension and had the advantage that it could be readily recognized in the gut after sectioning, and stages in the digestive breakdown of the corpuscles could be followed over the succeeding 8 h, until absorption was complete. Digestion could be carefully localized, and was found to take place in the lumina both of the stomach and of the digestive diverticula. Table II sets out the histologically visible signs of erythrocyte digestion over a period of 10 h after feeding.

For the first 30 min after feeding, the cytoplasm of the erythrocytes stained blue with azan. With the onset of digestion, the cytoplasm was attacked first, and during the first 2 h the outlines of the cells became more irregular until, at length, the nuclei were alone recognizable. Azan staining had the great advantage that its reaction with the erythrocyte changed from light blue to yellowish brown or orange as digestion proceeded. Eroded corpuscles were

TABLE II. PROGRESS OF DIGESTION OF DOGFISH ERYTHROCYTES BY *LASAEA RUBRA* DURING 11 H, AFTER FEEDING WITH A PALE SUSPENSION OF BLOOD IN SEAWATER

(Animals were removed after feeding for various lengths of time, and in some experiments were kept in filtered sea water for varying times before fixing. Fixed Bouin's, stained azan.)

Specimen no.	Time in blood suspension	Time in filtered sea water after feeding	Condition of erythrocytes after azan staining of sections	Condition of digestive gland (see pp. 571 *et seq.*)			
				I	II	III	IV
1	30 min	—	Many in the oesophagus and stomach, all intact, with cytoplasm blue. Majority in diverticula intact and blue, though a few eroded, and cytoplasm yellowish	×	×	—	—
2	30 min	—	Only a few in diverticula, none digested. Many in stomach not digested.	× ×	×	—	—
3	1 hr	—	Majority in stomach and diverticula undergoing digestion, cytoplasm orange yellow, margins usually much eroded. Nuclei persist longest. Final stage is of dispersion of yellowish granular cytoplasm released from the cells. Free nuclei visible (See Fig. 2)	× ×	—	—	—
4	1 h 50 min	—	All in stomach staining yellow to orange; much erosion in diverticula, and dispersion of amorphous yellow material. Yellowish droplets appearing in distal cytoplasm of digestive cells	×	×	×	—
5	1 h 50 min	—	Very eroded in diverticula, intensely orange. Much diffused yellowish material in lumen, some being absorbed by digestive cells. No free nuclei detected	× ×	—	—	—
6	2 h	1 h	All now in the diverticula, much more digested than previously; very few intact, but released yellowish material freely taken up along upper third of digestive cells	×	× ×	—	—
7	2 h	1 h	Much diffuse yellow from digestion. No whole corpuscles. Digestive cells absorbing	×	× ×	×	—
8	2 h	1 h	A few erythrocytes, almost wholly eroded in diverticula, and a great deal of diffuse yellow	× ×	×	—	—
9	2 h	1 h	Identical with (8)	×	× ×	—	—
10	2 h	2 h	No corpuscles and little diffuse yellow material in lumina of diverticula, but the upper half of the digestive cells loaded with orange-red droplets. Digestion and absorption appear complete	—	× ×	×	—
11	2 h	3½ h	A few areas of yellowish material remain in the lumen, but digestion is mostly completed. Orange-red granules now deeper in the digestive cells, with a superficial layer of blue-staining vacuoles (? material absorbed after blood)				
12	2 h	3½ h	Similar to (11)	—	× ×	× ×	—
13	2 h	8½ h	No trace of yellowish material in lumina of diverticula. Digestive cells and cut off spherules filled with reddish brown vacuoles	—	× ×	× ×	—
14	2 h	8½ h	Identical with (13)	—	× ×	× ×	—

always yellowish brown, and after the complete breakdown of the cytoplasm, large amounts of yellowish granules appeared in the lumen. Absorption by the digestive gland could also be traced by staining with azan. At 3 h from first feeding, droplets of yellowish material were being taken up, after 4 h the vacuoles of the upper layer of the epithelium were strongly reddish brown after azan staining. After 5½ h these vacuoles lay more deeply and there was a superficial layer of light-blue staining vacuoles, which had evidently arisen from material later absorbed (see Fig. 2).

Azan staining may not be ideal for the tracing of erythrocyte digestion, since its coloration is apt to be unpredictable and uncertain in interpretation. In *Lasaea*, however, orange or reddish stained digestive vacuoles were never obtained save after feeding with blood, and the method by good fortune allowed digestion and absorption to be reliably traced. Another staining technique that might be attempted after digestion of erythrocytes is the Prussian blue detection of iron after its possible release as 'haemosiderin'. While haemoglobin specific stains are sometimes lacking in definiteness, the writer has found Van Gieson's to be a general stain that picks out haemoglobin rather distinctively (see also Dunn & Thompson, 1945).

Although occurring *in vivo*, these experiments were subjected to as careful as possible control. It was suspected that the cells might have been disintegrated by the mechanical action of the surrounding mucus or ruptured by the effect of the lower pH of the stomach contents, rather than by enzyme action. Cells were therefore examined that had passed through the mucus of pseudofaeces, and were found to show no erosion. Comparison was made with blood cells in other locations in the gut of ciliary feeders where enzymes were not active. It was impossible to measure the stomach pH of *Lasaea*, but this was assumed (see Yonge, 1925), to be somewhat greater than 5. Suspensions of dogfish erythrocytes and of *Phaeodactylum* were therefore immersed in salt solutions isotonic with sea water and buffered at pH 5·3, 5·5 and 5·9. Inspection at hourly intervals over the period of the experiments never showed any evidence of rupture or disintegration. Smears of dogfish blood were also subjected to digestion by mammalian trypsin at pH 7, after which the erythrocytes showed erosion in a similar way to those fed to *Lasaea*.

References to 'extracellular digestion' in this paper must clearly be taken to imply digestion in its widest sense. Preliminary digestion of relatively large cellular particles in the stomach proceeds at least as far as the breakdown of the cell wall; the cell contents are thus liberated in finely divided form, able—with or without further digestion—to be ingested by the free surface of the digestive cells. No one to-day would doubt that the digestive gland has an important function of intracellular digestion.

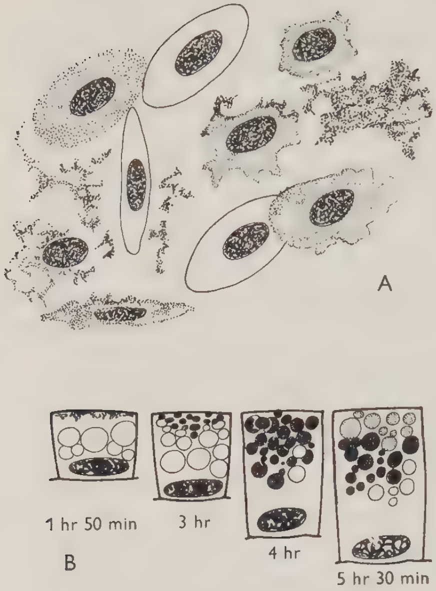

Fig. 2

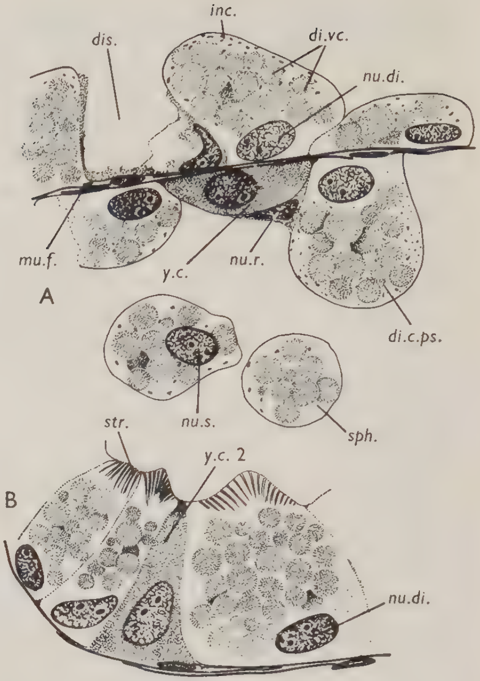

Fig. 3

Fig. 2. A group of dogfish erythrocytes in the lumen of the digestive diverticula of an animal that had been fed for 1 h with a suspension of blood corpuscles, and fixed in Bouin's fluid immediately. Erythrocytes with clear cytoplasm stained blue in azan, the nuclei red. After digestion an orange colour was shown by the cytoplasm, stippled in the figure, of both the intact and the eroded erythrocytes. Digestive cells, represented diagrammatically at 1 h 50 min, 3 h, 4 hr, and 5 h 30 min, respectively, after feeding with a suspension of blood. Absorbed material staining orange or brown with azan is represented black.

Fig. 3. (A) Portion of the wall of two adjacent tubules of the digestive gland. The digestive cells are at the beginning of the fragmentation stage, following absorption, and two spheres released into the lumen are shown in the lower part of the figure. Fixed Flemming's without acetic. (B) A group of cells from a tubule of the digestive gland during or after absorption, but before fragmentation. The striated layer at the free border is well shown. Fixed Bouin's fluid. Both drawings are from sections stained with azan. *di.c.ps.*, digestive cell which has become pseudopodial prior to the formation of a sphere; *di.vc.*, vacuole of absorbed material within a digestive cell, blue-staining with azan; *dis.*, space left in the epithelium after the discharge of a sphere; *inc.*, small inclusions, red-staining in azan, in the cytoplasm of a digestive cell; *mu.f.*, muscle fibre; *nu.di.*, nucleus of a digestive cell; *nu.r.*, nucleus, (?) remaining after discharge of a sphere, displaced to the surface of the epithelium; *nu.s.*, nucleus of a free sphere, lying in the lumen; *sph.*, sphere within the lumen, with no nucleus apparent; *str.*, layer of striations at the free surface of the digestive cell; *y.c.*, young cell; *y.c.* 2, young cell, compressed to a narrow shape between adjacent digestive cells.

THE CYCLE OF THE DIGESTIVE GLAND

The structure of the digestive gland was studied in two ways: by the examination of stained serial sections, of which many were cut; and by the viewing of the whole gland in living condition immediately after removal from the animal. For this purpose digestive glands were isolated from other tissues, quickly mounted in sea water, lightly covered and immediately sketched under $\frac{1}{12}$ in. oil-immersion objective. For quickness and accuracy this method was by far the more useful: the whole gland could be carefully scrutinized, while avoiding all fixation artifacts and allowing reliable interpretation of the living cell. This is another of the advantages of working with a small animal. Furthermore, large numbers of preparations could be made with no laborious procedure.

On the whole, the best stained preparations were obtained after fixing with Flemming's without acetic. Though penetration of Flemming's was usually poor and microanatomy distorted, yet in cell detail the best preparations with Flemming's were the finest of any obtained. Both aqueous Bouin's and Susa were found suitable for microanatomy, and both penetrate well. In spite of what is sometimes alleged against it as a cytoplasmic fixative, I have not found Bouin's, in this or in other molluscs where I have used it, much inferior to Flemming's. It has the great incidental advantage that it perfectly removes the shell. Ten per cent. formalin always gave poor results with *Lasaea*, penetrating badly and yielding poor staining. Heidenhain's azan was used for staining throughout.

The tubules or follicles of the digestive gland are lined wholly with glandular cells, which, as usual in bivalves, are essentially of one kind. No cilia were found in the tubules anywhere beyond the exit of the diverticula from the stomach, and in this feature *Lasaea* differs, probably again on account of its small size and functional simplification, from the numerous lamellibranchs recently studied by Owen (1955).

The digestive cell is a versatile structure and passes through several forms during its history. We should first refer to the nests of small cells, which correspond to the 'crypts of young cells' described by Yonge (1926*a*, *b*) and by Owen (1955). These cells in *Lasaea* occupy the tips of the older tubules of the digestive gland, and have a very different appearance from the absorbing cells. They are coloured pale yellow in life and appear to lack all forms of visible inclusions. They generally form groups of half a dozen or more cells at the tubule tip (see Figs. 5 and 6); and small spherical clusters of young cells are usually also to be found, forming smaller club-shaped branches that evidently represent the rudiments of new tubules. Owen describes the 'young cells' in eulamellibranchs in general as extending from the tubule tip in two or more tracts or 'crypts' along the whole length of the lumen of the tubule. Isolated young cells could often be seen in *Lasaea*, mingled with the more mature absorbing cells, along the length of the tubule, but their most usual position is always in clusters at the tip. In correlation with the small size of the animal, and the relatively small number of cells constituting the whole tubule, replacing cells would seem as a rule to be concentrated at the tip of the tubule rather than diffusely scattered. A well-known parallel to this distribution of young cells is found in the nests of cells at the tips of the digestive tubules in the crayfish, *Astacus*, described and figured by Jacobs (1928).

Difficulty was encountered in *Lasaea* with the fixing and staining of the contents of the terminal cells. Oldfield (1955) states that, on fixing with Susa, the cell contents contract from the periphery and form a sphere of heterogeneous consistency. With Bouin's and Flemming fixation, the writer's results were generally unsatisfactory, the cells appearing as almost empty vesicles with their cytoplasmic contents barely recognizable or wholly shed. The isolated young cells dispersed irregularly among the digestive cells (see Fig. 3, *y.c.*) were, however, usually well fixed, and their cytoplasm stained pinkish brown with azan.

Oldfield (1955) remarks upon the great difference in appearance of the terminal cells from the absorbing cells, and gives evidence for the presence of two distinct types of cell in the digestive tubules of *Lasaea*. A short statement is thus necessary of the present writer's reasons for adopting a different interpretation on this point, and for taking a unitarian view of the terminal cells, as the youngest stages of a single versatile cell type, the absorptive and digestive cell. In specimens examined during this work, it seemed that the cell contents, 'of semifluid yellow material', rather than forming a vacuole, constituted in fact the whole of the cytoplasm of the cell, uniformly light yellow coloured and devoid of any stainable inclusions. As Oldfield points out, there is no response to tests for calcium, glycogen, fats or lipides. In the examination of digestive glands dissected living, these terminal cells can be shown to be wholly similar in form and appearance to the light-coloured 'young cells' interspersed with mature cells along the length of the tubule. Furthermore, in digestive glands examined alive immediately after feeding, pale-coloured cells in the terminal groups were seen to be absorbing droplets of pigmented food material along their free surface, while otherwise identical in appearance with non-absorbing cells alongside them. At times the whole cell group at the tip of a tubule was found to be carrying out absorption. In other specimens, soon after feeding, a single non-absorbing 'young cell' alone remained at the tip of the tubule; all its neighbours had been pressed into service as absorbing cells. This was the picture most generally seen within an hour or two of feeding; by contrast, in animals that had remained dry for some hours, the most conspicuous feature of the almost colourless digestive gland was the appearance of small rudimentary tubules, with no contents in their lumina, and composed wholly of 'young cells' that had not absorbed (see sketches from life in Fig. 6).

The position of young cells at the tip of the tubule is in agreement with Owen's results on a wide range of eulamellibranchs, and also, as was pointed out above, with the condition in the digestive gland of *Astacus*. It would be desirable, but was not attempted during the present work, to investigate the incidence of mitotic divisions in these cells in *Lasaea*. Yonge (1926*a*) found numerous mitotic figures during histological studies of similar cells in other lamellibranchs. It is remarkable that the terminal cell groups should fix and stain so poorly as compared with the similar cells irregularly scattered among mature digestive cells. One must suppose that the uniform, semi-fluid cytoplasm of the groups of young cells is peculiarly susceptible to disruption by the tensions set up by diffusion currents in fixing and dehydrating. The isolated cells, surrounded by mature absorbing cells packed with contents, would thus be better protected against distortion arising during fixation.

The young cells are at first typically dome-shaped or pyramidal (see Figs. 5 and 6) and bulge from a flat base towards the lumen. Although they were never seen to be ciliated in *Lasaea*, either by the present writer or by Oldfield (1955), they seem with little doubt to be cells of the same type as were found in other lamellibranchs (see Owen, 1955; Potts, 1923) to bear long lash-like cilia at this stage of their history.

The digestive cells do not long remain dome-shaped or colourless, and the first transformation seems to be brought about by the flow of food material into the tubules. The lumina now become widely distended, and—though certainty is difficult on this point—the impression is gained that the young cells constituting some of the tubules are actually stretched by the mechanical pressure of contents, and that as the tubule is enlarged, so each cell becomes wider based and flattened. The surface appears at this stage to absorb material freely from the lumen, and there is often a continuity of staining between the cytoplasm of the cell and the contents of the lumen. At the same time there is a gathering of yellow vacuoles in the cytoplasm.

Absorption was studied by several experiments with natural and artificial foods. A suspension of colloidal graphite ('Aquadag') was fed to the animal, by mixing it with *Phaeodactylum*. After 2 h, absorption was freely in progress, and the surface of the digestive epithelium was dark grey. Closer examination revealed that graphite particles lay freely in the superficial cytoplasm, and were not at this stage contained in vacuoles. After feeding with *Phaeodactylum* alone for 1 h, immense numbers of tiny colloidal droplets could be detected both in the lumen and in the cytoplasm surrounding the vacuoles. They were being rapidly absorbed by the cells. Pigment introduced with food organisms, as well as probably indigestible waste materials, seems to aggregate in the vacuoles. After feeding for 2 h on *Phaeodactylum* made pink with neutral red, a pink coloration was found in the vacuoles in the upper half of the cell, those at the base remaining yellow. In addition, minute red-stained granules lay freely in the cytoplasm between the pink vacuoles. These probably represent particles derived from the preliminary extracellular digestion of *Phaeodactylum* and now being taken up by the cells. A similar absorption of pigment by the vacuoles could be detected after feeding with a culture of *Dunaliella*, a thin-walled chlorophycean flagellate (6–12μ) (Plymouth no. 83). After $1\frac{1}{2}$ h feeding a long rope of flagellates was found attached to the head of the style and each digestive cell showed a conspicuous absorption of chlorophyll in all the vacuoles of its superficial half. Preliminary digestion of *Dunaliella* took place extracellularly. The cells—as with *Phaeodactylum*—are much too large for direct ingestion; in one case where the lumen of a tubule was clogged with undigested flagellates no ingestion took place.

In addition to the absorption of graphite and plant pigments, the ingestion of soluble and insoluble iron saccharate by the digestive cells of *Lasaea* has been well demonstrated by Oldfield (1955).

Absorbing cells increase rapidly in height to columnar or bulging form (Fig. 3). During absorption the cytoplasm becomes loaded with conspicuous orange-yellow vacuoles which impart their colour to the living digestive gland. Such vacuoles seem to occur very widely in the molluscan digestive gland. They are essentially similar in *Lasaea rubra* to those recently described in the ellobiid pulmonates by Morton (1955*a*) in an account of the cyclic changes of the digestive gland. Their staining reactions in various Mollusca seem very uniform. They are coloured blue after azan (except in some rather exceptional cases, as after feeding with blood, p. 569); they become green with Masson's trichrome and do not stain at all with haematoxylin, or with the mucus stains alcian blue or mucicarmine. They appear to be loaded with lipoid material which they probably store and which is responsible for their strong staining reactions with Sudan scarlet and with osmic acid. The vacuoles are not, however, simply droplets of lipoids: they have a permanence in the cell quite distinct from their occupation by fatty inclusions, and after treatment with acetone or ether they can be seen to persist as colourless spheres. Staining reactions with azan and with Masson's stain are well elicited in sections from which stored fat has been removed by routine treatment.

In the pulmonate *Otina* (Morton, 1955*b*) these vacuoles were studied in their 'empty' colourless condition towards the base of the cell, and were shown to be covered by a surface layer or membrane, stainable with thionin, but with no other stains tried. Attempts to detect it in *Lasaea* failed. This layer has many of the properties of that described by Barrington (1951) surrounding the 'chromophobe bodies' in the islet cells of the frog's pancreas. Colourless vacuoles are present in the digestive cells before the ingestion of food, and the writer earlier suggested (1955*a*) that the cell enzymes might be contained in or around them, or absorbed on the 'surface layer' when it occurs. There is as yet little positive evidence of this, and it would be of interest to examine further the enzyme localization of the digestive cell. The small size of *Lasaea* does not, however, make it ideal for such tests.

The vacuoles of the digestive cell owe their coloration to mixtures of pigments taken into the cell from the materials fed upon. These pigments, originating in phytoplankton and fragments of plant detritus, included large amounts of chlorophyll. For extraction of the total pigments from *Lasaea*, pale or colourless low-tidal individuals were selected, with a minimum of algal contamination of the shell. The spectral curve obtained after extraction with methanol of a greenish yellow solution from the soft parts is shown in Fig. 4. With peaks at 670, 535 and 420, it corresponds fairly closely with that of chlorophyll. Examination of macerated digestive glands with the spectroscopic microscope was difficult because of the animal's minute size; the 670 band, however, seemed fairly clearly identifiable. Pigments of the fucoxanthin type might also be expected to appear in the digestive gland, deriving with chlorophyll from the phytoplanktonic food (see Heilbron, 1942), and these are

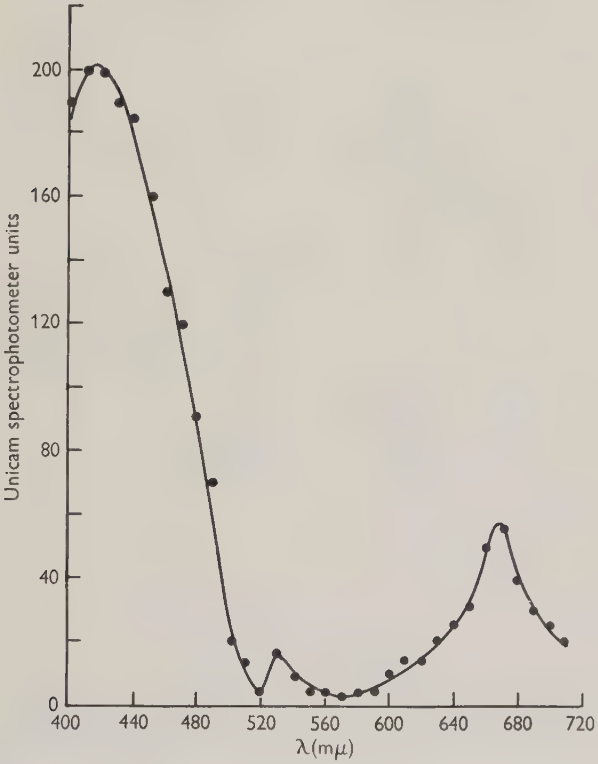

Fig. 4. Light-absorption curve obtained from the pigments extracted from the digestive gland, measured by the Unicam absorptiometer.

probably chiefly responsible for the yellow coloration. No attempt has yet been made to extract separately the pigments present in *Lasaea*, and in the absorption curve shown in Fig. 4, fucoxanthin bands at *c.* 450 and 480 would—if present—be concealed (Strain, Manning & Hardin, 1944).

During the absorption of food the free border of the digestive cell may often be obscured by the passage of particles from the lumen. At other times, following soon after absorption, its free surface possesses a narrow border of fine striations, and when the surface of the cell bulges towards the lumen, these are sometimes clustered radially to form a fan. The striations are identical with those described by the present writer in *Struthiolaria* (1951) and in the ellobiid pulmonates (1955 *a*). It was suggested that in the ellobiid *Leucophytia* they might represent the 'pore canals' of Baker's (1942) free cell border in the vertebrate intestinal cell. Their stage of occurrence in the digestive cell makes it likely they may represent an absorptive pathway. Yonge (1926 *a*) speaks of this layer as a fine cuticle in protobranchs and filibranchs, and Owen (*in litteris*) confirms its presence in other lamellibranchs he has studied.

As the digestive cell increases in volume, its free border becomes bluntly pseudopodial and proceeds to bulge into the lumen. Two or three hours after cessation of feeding the contents of each of the bulging cells become nipped off into the lumen in the form of a small sphere, filled with yellow vacuoles. Some of these spheres, but evidently not all, contain the cell nucleus, and they would then correspond to the 'fragmentation phagocytes' formed in this way in gastropods. Many of them, however, appear to be non-nucleated, and the basal nucleus is left behind, where it may later be squeezed out or pressed up to the free surface of the epithelium by the encroaching of absorbing cells at either side. The spheres move about freely in the lumen of the tubules. The wall of diverticulum is distinctly though finely muscular, and can sometimes be seen to give a series of short contractions, squeezing the spheres and other contents of the diverticulum toward the stomach.

After removal of the animal from water for more than 10 h (see Fig. 5) the digestive tubules show a majority of empty cells, most of the contents having passed out as spheres. The digestive gland is studded with clusters of new tubules, consisting of young cells that have not yet absorbed.

At most times, spheres can be identified in the lumen (see Fig. 3 *a*). They are generally present in some numbers during the absorbing phase of the digestive gland (Fig. 5 *a*), though their time of formation appears always to be at the final stage of the cell's history, after absorption has been completed. They mark the end of the life of the cell, and their formation is a type of holocrine 'secretion'. The production of free spheres from the digestive cells has generally been regarded in lamellibranchs as a form of excretion. By this means, much pigmented material and indigestible remains are returned to the stomach for disposal as faeces. Owen (1955) has shown in detail how the arrangement of the typhlosole of the stomach and the excurrent tracts of

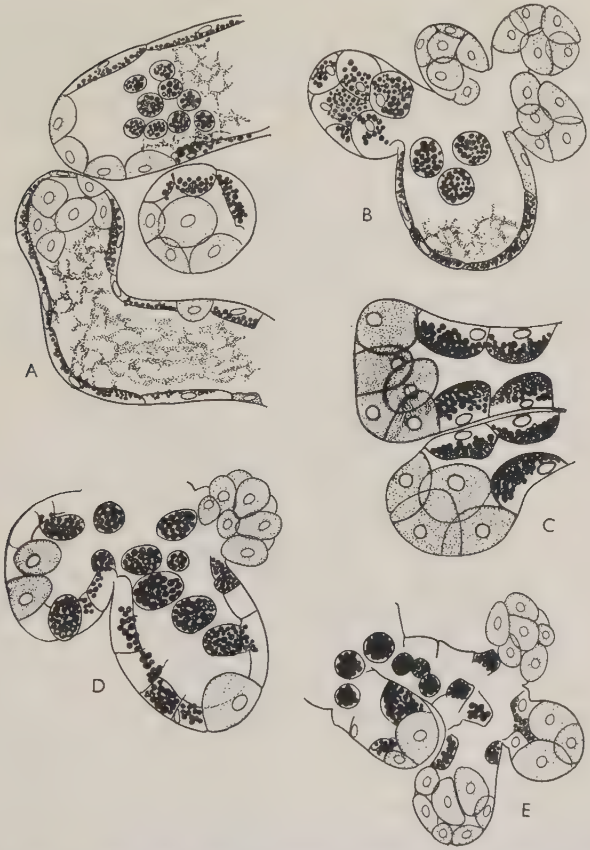

Fig. 5. Sketches made from living digestive glands, showing the features typical of various stages in the cycle. The portions of tubules illustrated are drawn from preparations examined as transparent objects under 1/12 objectives, the cover-slip resting lightly to prevent distortion of the cells. Young cells are present singly, in groups, or as young tubules and are lightly stippled in the figures; the coloured vacuoles in the mature cells are represented black. (A) Stage of absorption, with wide stretched tubules and flattened cells. Some free spheres are still present intact within the lumen of one of the tubules. Fed with *Phaeodactylum* for 1 h and examined immediately. (B) A later stage, from an animal exposed for 3 h after tidal withdrawal. The sketch shows three immature tubules composed of young cells, and, rather untypically for 3 h exposure, a tubule containing one young, non-absorbing cell, and the rest of its cells stretched flat and taking up contents from the lumen. (C) Stage of intact cells filled with absorbed contents, from a specimen exposed for $3\frac{1}{4}$ h after feeding with *Phaeodactylum*. The two tubules show very typically the appearance of groups of young cells at the rounded tips. (D) Stage of fragmentation of digestive cells, to produce free spheres. The majority of the digestive cells have discharged their contents, and are represented by colourless spaces. One cell is shown as a pseudopodial projection about to be cut off into the lumen. (E) Stage of fully discharged epithelium, from a specimen exposed for more than 12 h after feeding on *Phaeodactylum*. The majority of the cells are represented by empty spaces, and there are numerous spherules lying freely in the lumen. Three immature tubules are shown, composed of colourless young cells.
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the digestive diverticulum secure the direct transfer of rejected material to the intestine. In the simple stomach of *Lasaea* no such mechanism appears to be developed, and—from such observations as could be made—intact spheres were never recognized in the faeces. There is much evidence they disintegrate in the lumen of the digestive gland or the stomach. When they do so, two sorts of contents are released—yellow pigmented vacuoles, and the residual cytoplasm that lies between the vacuoles. Numerous yellow vacuoles are found in the lumen, and at the time of release of spheres, a small number of golden brown, oily droplets appear in the stomach. These are always much larger than the cell vacuoles, and they seem to consist of globules of pure oil or fat. They are probably formed by the coalescence of the oil vacuoles of the disintegrated spheres.

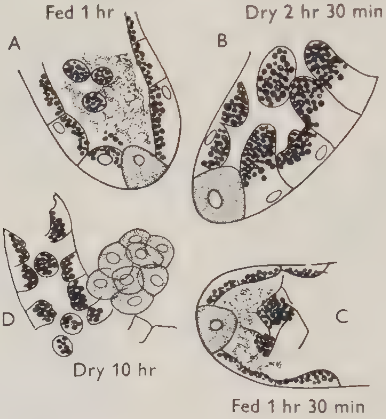

Fig. 6. Sketches from living preparations showing the single remaining 'young cell' at the tip of the tubule, after the surrounding cells have been engaged in absorption for 1 h or longer (A, B, C). Comparison should be made with (D) which shows a cluster of 'young cells' forming the rudiment of a tubule, in a specimen that has been several hours exposed; and with the group of young cells forming the whole tip of a tubule in Fig. 5 (*c*).

These large droplets were occasionally found in living preparations in such proximity to a recently discharged digestive cell as to suggest that they had been formed *in situ* by the coalescence of vacuoles before discharge. Such large vacuoles would then resemble those of the 'excretory cells' found in the digestive gland of gastropods. They were never found in sections of *Lasaea*.

Disintegration of the spheres may also release traces of free enzymes for extracellular digestion. It is suggested that these enzymes originate in the residual cytoplasm of the cells after the vacuoles are shed, and are responsible for the preliminary digestion of flagellates, *Phaeodactylum* cells and dogfish blood corpuscles shown to occur before absorption by the digestive gland. In other lamellibranchs, the free enzyme action detected in the stomach has been suspected to be due to the action of amoebocytes. In *Lasaea*, there would appear to be a different mechanism whereby food is able to be broken down by preliminary digestion to particles minute enough to be absorbed by the digestive cells. The digestive cycle is primarily one of absorption and excretion; but from the contents of previously fragmented cells there appear to remain sufficient free enzymes to initiate a first phase of extracellular digestion.

TIDAL RHYTHM

The sequence of activity of the digestive cells was investigated by the examination of 225 animals, at known times after feeding or exposure by the tide. Some of these were fed experimentally, with *Phaeodactylum*, *Dunaliella*, colloidal graphite ('Aquadag') or dogfish erythrocytes. Others were collected at regular intervals from the field, and examined after natural feeding. Examination was by dissection of the living digestive gland, as described above (p. 573) or from stained serial sections. Table III presents in condensed form the methods used in this survey, and its results; while Fig. 7 shows graphically the condition of the digestive gland from hour to hour during feeding and subsequent exposure. Three stages of activity of the digestive gland have been recognized, namely, (I) *absorption*, with flattened epithelial cells of low height and widely distended tubules; (II) *digestion*, with the majority of cells fully loaded and columnar; (III) *excretion*, with the production of free spheres by rounding off and liberation of the epithelial cells. 'Secretion' may be regarded as a subordinate activity taking place together with excretion. A further condition (IV) may be recognized where the epithelium is almost completely discharged of coloured cells, after several hours' exposure, and the whole digestive gland is pale or colourless.

Tables III and IV and Fig. 7 disclose a periodicity of the digestive gland, in relation to feeding and exposure. Like the presence of contents in the stomach and the size of the crystalline style, this is under ultimate tidal control. It is thus possible to speak of a tidal rhythm in the digestive system of *Lasaea*. Processes that may take place more or less continuously in bivalves mostly covered by the tide are, in high-tidal *Lasaea*, compressed into the short period of 3 or 4 h out of the 12, the period to which filtering is restricted.

There is no period when it is possible to say that all the cells of the digestive gland are at a particular stage, and it is perhaps unlikely that all the cells or tubules of a digestive gland ever take part together in one cycle of activity. Nevertheless, it is possible to recognize a well-marked *predominance* of activity, during various times of feeding and exposure. Thus, immediately after feeding the cells are with few exceptions at the flat, actively absorbing stage. And later in the tidal cycle, subsequent stages can be very satisfactorily followed, each having a maximum period, varying in length with the duration and amount of feeding. Successive phases overlap, and in all cases young cells are present in clusters at the tips of the tubules.

TABLE III. CONDITION OF DIGESTIVE GLAND AFTER FEEDING

(The condition of the digestive gland is given in relation to time of feeding or exposure in 232 specimens of *Lasaea rubra*. The stages recognized are (I) absorption, (II) intracellular digestion, (III) formation of spheres, (IV) post-spherulation (see pp. 579 *et seq*.). A bold numeral indicates a *predominance* of a given stage. A bracketed numeral indicates a stage that was also frequent.)

Series I (4/54). Fed *Phaeodactylum* 1½ h, transferred to filtered sea water. Fixed at intervals shown. Examined in stained sections. (20 specimens.)

Hours after feeding	I	II	III	IV
		(numbers of specimens)		
1	8	3 (3)	1	.
2	(2)	5	1 (2)	.
3	1 (1)	3 (2)	1 (2)	.

Series 2 (4/54). Fed erythrocytes up to 2 h, transferred to filtered sea water, and fixed at intervals shown. Examined in stained sections. (43 specimens.)

Hours after feeding	I	II	III	IV
.	**12** (1)	4 (10)	(3)	.
1	1 (2)	5	(1)	.
2	.	2 (4)	1 (4)	.
3½	(2)	5 (1)	(3)	.
6½	1	2 (2)	2 (2)	.
8	(1)	4 (2)	3 (3)	(1)

Series 3 (4/54). Fed *Peridinium trochoideum*, 1¼ h. No digestion. Fixed immediately. (5 specimens.)

I	II	III	IV
1 (2)	5	1	.

Series 4 (4/54). Fed *Isochrysis galbana*, removed into filtered sea water. Fixed after intervals shown. (5 specimens.)

Hours after feeding	I	II	III	IV
7	1	1 (1)	1 (1)	.
8½	1	1	(1)	.

Series 5 (4/55). Fed 'Aquadag',* *Phaeodactylum* with neutral red,† and *Dunaliella*,‡ for times shown, and examined alive immediately after feeding. (7 specimens.)

Hours fed	I	II	III	IV
2*	3	.	.	.
2†	2	(1)	.	.
2‡	1 (1)	(1)	.	.
3‡	.	.	1	.

Series 6 (7/54). Fixed in the field daily over 8 days. Examined in stained sections. (43 specimens.)

Hours after feeding	I	II	III	IV
½	**5**	(4)	.	.
¾	1 (2)	5 (1)	3	.
1	2 (2)	3 (2)	1)	.
3	(1)	**2**	1 (2)	.
3½	1 (1)	2 (1)	1(1)	.
6	(2)	6	(1)	.
7	1 (1)	4 (2)	1 (3)	.
7½	(1)	4 (2)	3 (1)	.

Series 7 (8/54). Fed *Phaeodactylum* for times shown and fixed while still feeding. Examined in stained sections. (38 specimens.)

Hours fed	I	II	III	IV
2½	6 (4)	8 (3)	1 (5)	.
2¾	(1)	(3)	4 (1)	.
3½	2 (4)	3 (2)	1 (2)	.
4	.	.	1 (2)	.
5	1 (1)	4 (1)	2 (4)	.

Series 8 (8/54). Examined alive after feeding with *Phaeodactylum* or after exposure in the field for the periods shown. (42 specimens.)

Hours after feeding	I	II	III	IV
1¾	(1)	1 (2)	2 (6)	1 (3)
2½	.	.	1 (5)	(4)
3½	(1)	1	2 (2)	1 (1)
4½	.	(1)	4 (2)	2 (2)
5⅜	.	.	2 (3)	1 (4)

Series 9 (4/55). Examined alive after feeding with *Phaeodactylum* or after exposure in the field for the periods shown. (42 specimens.)

Hours fed	I	II	III	IV
1	2	.	(1)	.
1–2	1	.	.	.
2–3	(1)	1 (1)	.	.
3–4	2	(2)	(2)	.
4–5	.	2 (1)	1 (2)	1
Hours since feeding	I	II	III	IV
2–3	.	(5)	5	1 (1)
3–4	1 (1)	2 (4)	3 (4)	4 (3)
4–5	.	1 (2)	5	2
12–13	.	.	2 (6)	9 (1)

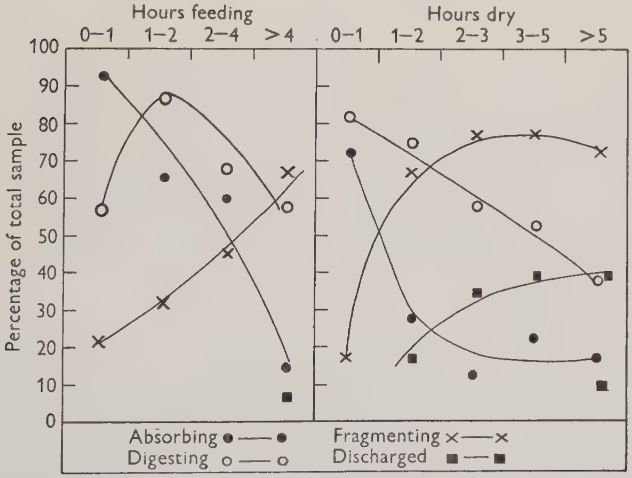

Fig. 7. Graph of the data set out in Tables III and IV showing the relation between the condition of the digestive gland, and the hours for which the animal had fed (left) or the hours for which it had remained exposed, following upon feeding (right).

Fig. 7 makes a comparison between two series of experiments in which animals were rather differently treated.

In the first series (results shown on the left) animals had been experimentally fed with *Phaeodactylum*, or with dogfish erythrocytes, in much greater abundance than that of natural food. After 4 h, the stages of 'absorption' and 'digestion' have generally been passed through, and 'fragmentation' predominates. Although the animals were still immersed in a suspension of food particles, the digestive cycle had—after overfeeding—advanced to a further stage than is normally found when animals in the field are left dry by the retreat of the tide. Animals which had spent 2 h feeding in natural conditions in the field were examined in a second series of experiments, and the results are shown on the right of Fig. 7. The speed of the digestive cycle is thus controlled by the length of time of submersion and of feeding. The cycle in upper tidal *Lasaea* may be not an intrinsic one, but one imposed merely by the external factor of feeding periodicity. It can be experimentally varied, and an actively absorbing phase of the digestive cells can at any time be produced by experimental feeding. In low-tidal *Lasaea*, and probably in most other lamellibranchs, the relation of the digestive cycle to the tidal rhythm must be much less clear-cut.

TABLE IV. CONDITION OF DIGESTIVE GLAND

Percentage of occurrence of stages I to IV of the digestive gland, in relation to time after feeding or exposure, from results obtained with 225 animals. (In many cases a single animal is represented under more than one stage, and the calculations have been made by adding together the bold and bracketed entries in Table III.)

		Percentages			
Hours	Total no. of animals	I	II	III	IV
Fed					
0–1	14	93	57	21	·
1–2	9	66	89	33	·
2–4	33	60	69	48	·
Over 4	13	15	61	69	8
Exposed					
0–1	26	73	81	19	·
1–2	25	28	76	68	16
2–3	17	12	59	77	35
3–5	39	21	56	78	38
Over 5	49	18	39	72	39

Fig. 8 sums up in a diagram the probable course of the digestive cycle of *Lasaea rubra* in relation to the tides. A tidal level was chosen at which there is 9 h exposure and 3 h submersion at normal tides. From Table I food is seen to have left the stomach within the first hour after tidal withdrawal. After 1–2 h faeces lie in the middle intestine, and after 8 h exposure the last remains of faeces are in the rectum or discharged into the pallial cavity. The style is largest while food is in the stomach, drawing in the food string by its rotation. During exposure it becomes too small to be rotated in its sac, but is probably not often lost altogether. It is rapidly rebuilt during the first hour after the tide returns. The sequence of the four phases of the digestive gland extends throughout the 12 h of the tidal period, and is evidently controlled primarily by the presence of food in the stomach.

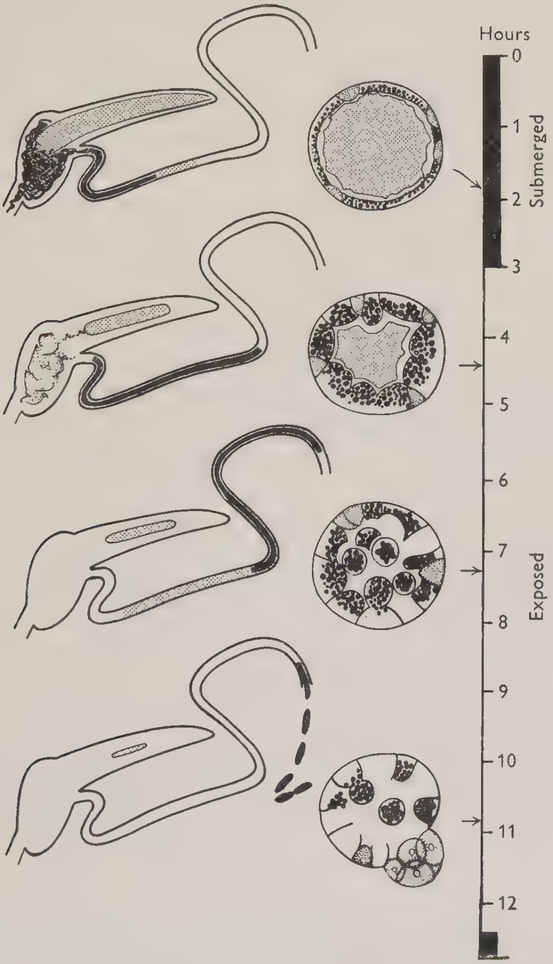

Fig. 8. Diagram showing the relationship between the periodicity of the digestive system, and the submergence or exposure of the animal by the tides. The outline drawings of the stomach, style sac and intestine indicate the size of the crystalline style, and the position of food in the various parts of the gut. The diagrams of digestive tubules, in transverse section, show the following stages: (I) absorption; (II) fully loaded cells, presumably with intracellular digestion in progress; (III) fragmentation, and formation of spherules; (IV) discharged epithelium, with spherules in the lumen, and a developing tubule composed of young cells.

Comparison may be drawn between the cycle of the digestive gland in *Lasaea rubra* and that of the digestive diverticula of the decapod crustacean *Astacus*, which was carefully investigated by Hirsch & Jacobs (1928, 1930). In decapods, the young cells forming the tip of the tubule give rise to two separate types of cell, secretory and absorptive. The secretory cells are under rhythmic control, which is speeded up greatly after feeding. Secretion is of the holocrine type, and ends the life of the cell, as does excretion by the formation of spheres in *Lasaea*. The cells are thus in both animals 'monophasic', by the definition of Hirsch (1931), and the whole gland shows a rhythmical working, since the time for restitution of the cell is greater than the time required for its discharge. The whole tubule in each constitutes a 'field of restitution'.

The cycle in *Lasaea* differs fundamentally from that of *Astacus*, in the occurrence of absorption and secretion as separate activities of one type of cell. In Crustacea (see Yonge, 1924) the action of the secreting cells precedes that of the absorptive cells. In the lamellibranch *Lasaea*, the secretory activity comes last of all, and is the final event in the life of the cell. This is because 'secretion' is an incidental event, added at the end of a cycle that is fundamentally based upon absorption and excretion. Traces of enzymes are provided in advance of the next arrival of food in the stomach, by the fragmentation of cells that have already passed through their absorptive and excretory phases.

Differences shown by *Lasaea* from the usual pattern of lamellibranch digestion are thus more apparent than real. The digestive cell has been shown to absorb and to carry out intracellular digestion, and excretory material is later shed into the lumen by fragmentation of digestive cells. From the cell size of *Lasaea*, direct ingestion of particles of the size of *Phaeodactylum* or most flagellates could not well take place. For this reason too, and also because of the concentration of digestion into a short time during each tide, phagocytic digestion may be inadequate. *Lasaea rubra* has thus, it is suggested, acquired a dependence on traces of enzymes shed by the digestive gland, in carrying out a preliminary stage of extracellular digestion.

SUMMARY

Lasaea rubra is a minute lamellibranch reaching in distribution almost to high-water spring tides. In some places, it is submerged for less than an hour at each tide and a tidal periodicity is thus imposed upon its feeding. The effect of this periodicity is reflected in the mode of action of the gut. The amount and position of food varies regularly with the state of the tide. The crystalline style undergoes a regular cycle, being partly dissolved and becoming vestigial at low tide, and being resecreted at the high tide. A sequence of four phases was established in the digestive gland, by the study of large numbers of animals, living and after sectioning, and after both natural and artificial feeding. While the animal is feeding, and shortly afterwards, finely divided material is absorbed into the cells of the digestive gland. Intracellular digestion then takes place, with the loaded digestive cell attaining its maximum size. Fragmentation of the digestive cells next occurs, with the discharge of rounded spheres, filled with vacuoles of rejected pigmented material. Finally, just before the tide returns, the digestive gland shows a discharged epithelium, with the digestive cells being replaced by young cells. Evidence is given of the close relation of this cycle to the tides. It is also shown that extracellular digestion of dogfish erythrocytes and phytoplanktonic organisms takes place within the stomach before absorption, and it is suggested that this is brought about by traces of enzymes in the fragmented spheres issuing from the digestive gland.

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ON THE BIOLOGY OF *CALANUS FINMARCHICUS*

IX. FEEDING AND DIGESTION IN THE YOUNG STAGES

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(Text-figs. 1–3)

Little is known about the food of the nauplii and early copepodite stages of copepods, although it has usually been assumed that the food particles must be smaller and perhaps more concentrated than for adults. A few observations have been made on the feeding of the young stages of *Calanus* in the laboratory, but none are available on feeding under natural conditions. It has been suggested (Marshall, Nicholls & Orr, 1934) that the success or failure of a brood of *Calanus* in the sea might depend on the presence of a rich food supply during its development. Experiments were therefore undertaken to find what organisms could be ingested by the nauplii and early copepodite stages, how much of the different foods could be digested, and how much water they could filter in a day.

METHODS

The use of food cultures labelled with radioactive phosphorus (^{32}P) enables an accurate measure to be made of the amount taken in, and the methods used in earlier experiments on feeding in adult and Stage V *Calanus* (Marshall & Orr, 1955*b*) were found to be useful for the younger stages also. When photosynthetic organisms are grown in a medium containing inorganic ^{32}P it is rapidly taken up and the culture can then be used in different concentrations to measure the amount filtered and the amount assimilated in a given time. A variety of food organisms of different sizes and belonging to different systematic groups was used. In each experiment the copepods were put in bottles filled with a culture of known concentration of the food to be tested and the bottles were then tied in dark cloth bags and attached to a wheel revolving slowly so that the culture was kept in suspension. All experiments were carried out in a cool aquarium. At the end of the experiment, which lasted from 16 to 24 h, the bodies, and sometimes the faecal pellets also, were removed and washed. They were then dried and exposed on a disc or planchette under the end window of a Geiger counter to measure the radioactivity. The results are shown as counts per minute. Specimens were also kept in filtrate from the radioactive culture to measure the uptake, if any, of ^{32}P in solution.

If faecal pellets were to be collected, the early stages of *Calanus* were kept in bottles which might be as small as 2 ml. capacity. For experiments in which faecal pellets were not collected, as many as 20 Nauplius III were put in a bottle of about 30 ml. capacity and the bodies were usually read in batches of about 10 at a time. The figures given for volume filtered by these are therefore averages. Occasionally, however, a Nauplius III was read singly and it was clear that this stage showed as much variation as any other. The later stages were usually read singly, although several might be kept in one bottle.

It is not so easy to judge the health of the early stages as of the adults. The amount of food taken up varied much in different experiments with the same stage, and it seems likely that those with the higher feeding rate represent the normal individuals. Experiments were therefore usually made with two species of food organism, one of which was known to be a satisfactory food. If this was eaten freely it was assumed that the copepods were in good condition.

Nauplius Stages I, II, III and sometimes IV were usually obtained from eggs hatched in the laboratory. The later stages were usually picked out from tow-nettings taken on the day of the experiment. They were commonest during the spring and summer in fine and medium tow-nettings, but a small number could be obtained throughout the autumn and even part of the winter. Nauplius I usually moults to Nauplius II in less than 24 h and Nauplius II to Nauplius III about 24 h later. Nauplius III remains as such for several days (Lebour, 1916) and mortality at this stage is usually high. The interval between moults is short in the early stages and at the end of an experiment some had remained in the same stage, some had moulted to the next and some were in process of moulting. Only the results from the first group are included in Table IV.

The food organisms were most often used in concentrations such as are found in the sea. Counts were made either with a haemocytometer or, more usually, in 0·2 ml. samples on a ruled slide. The number of cells present does not affect the calculation for the number of ml. filtered, but it can give an idea of the number of cells which the copepod ingests in a given time (Table VIII).

The following organisms were used:

Nannochloris oculata Droop (M. R. Droop's strain 66)
Monochrysis lutheri Droop (M. R. Droop's strain 60)
Dicrateria inornata Parke (Plymouth strain B)
Syracosphaera elongata Droop (M. R. Droop's strain 62)
Skeletonema costatum (Grev.) Cleve (Plymouth strain 106)
Chaetoceros decipiens Cleve (Plymouth strain 107)
Ditylum brightwellii (West) Grun. ex Van Heurck (Plymouth strain 110)
Prorocentrum micans Ehrenberg (Plymouth strain 97)
P. triestinum Schiller (Plymouth strain 98)
Peridinium trochoideum (Stein) Lemm. (Plymouth strain 104)

The dilution of the cultures was always made with water which had been filtered through a 'membrane' filter. This removed particles down to about 1 μ and so ensured that a negligible quantity of non-radioactive food was added. Filtering through filter-paper makes the water harmful to at least the early nauplius stages of *Calanus*. It was noticed that nauplii in Stage I mostly died or at least failed to moult when kept in paper-filtered water. The fine threads which come from the filter-paper probably get entangled in the nauplius limbs and prevent free movement. Rejecting the first portion of the filtrate and so reducing the number of fine threads led to an improvement in survival and moulting and a complete removal of these threads by filtering again through a 'membrane' filter resulted in normal survival and moult.

THE FILTERING APPENDAGES

Nauplii

Some observations on the feeding mechanisms of copepod nauplii have been made by Storch (1928). He states that the nauplius feeds like the adult, i.e. if the adult copepod is filter-feeding so is the nauplius, and, if the adult is predatory, the nauplius is predatory too. The limbs used in feeding by *Calanus* nauplii must be different from those used by the adult, for the maxilla, which is the main filtering appendage, does not appear until Nauplius VI and is then small and weak. Storch says that in *Diaptomus gracilis* nauplii the filtering is done by the long masticatory processes of the coxa and basipod of the antenna and mandible. In Nauplius I and II of *Calanus* these processes are short and weak and have no setules. As will be shown later, it seems certain that neither of these stages feeds. In Nauplius Stages III to VI the masticatory spines on the coxa and basipod of the antenna have developed further, are longer and stronger and lie close together. Each of the three (Fig. 1) has a double row of strong setules on the distal half about 10–30 μ long and 1·5–4 μ apart. These are the only setules present on the proximal

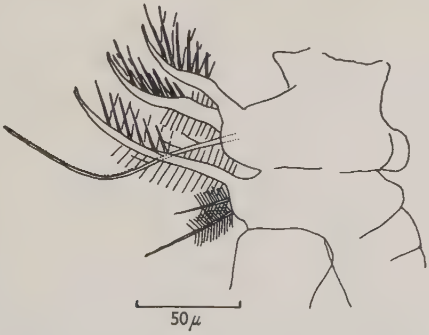

Fig. 1. Coxa and basipod of the antenna of Nauplius V.

coxal seta, but on the other two the lower halves have single rows of fine setules on each side. There is a long thin seta on the coxa and two shorter on the basipod, but these may play no part in filtration. It seems possible that the 'masticatory processes' are wrongly named and that their function is filtration rather than mastication. The function of the separate setae is not obvious.

The maxilla, which appears in Nauplius V as a knob, has in Nauplius VI almost the complete number of setae, but these are relatively very short and have no setules.

Copepodites

It may be assumed that in the copepodite filtration is done mainly by the maxilla (Cannon, 1928; Lowndes, 1935). This is a uniramous appendage bearing a series of setose endites. Following Gurney (1931), it may be taken as formed by a coxa bearing two endites and an external seta, a basipod bearing two endites, and a five-segmented endopod, the first two segments of which are produced as endites. The maxilla of *Calanus gracilis* (which differs only slightly from that of *C. finmarchicus*) has been beautifully figured by Giesbrecht (1892, Plate 7, fig. 17).

TABLE I. DISTANCE APART OF SETULES IN COPEPODITE STAGES (IN μ)

							Endopod			
		Coxa			Basipod		Segment 1		Segment 5	
	Stage	External seta	Endite 1	Endite 2	Endite 3	Endite 4	(Endite 5)	Segments 2–4	'Feather'	Others
C. finmarchicus	♂	—	5–12	4–8	6–9	5–11	4–16	5–21	4–5	6–17
C. helgolandicus	♂	2	5–9	8–9	8–10	8–11	12–17	11–19	7–9	13–22
C. finmarchicus	♀	2	2–6	3–6	3–7	3–8	4–7	5–14	3–5	5–14
C. helgolandicus	♀	2	5–9	6–9	4–9	6–12	7–13	11–19	3–9	12–20
C. finmarchicus	V	1–3	2–4	3–4	2–4	2–4	3–6	4–8	2–5	4–10
C. helgolandicus	V	1–3	3–9	4–7	3–8	4–10	3–15	5–17	3	7–20
	IV*	2	3	3–4	3–6	3–5	3–5	4–7	3–4	5–9
	III*	2	2–4	3–4	3–4	2–5	4–7	4–8	3–4	4–11
	II*	—	2–3	3–4	2–4	3–4	3–5	3–7	3–4	3–7
	I*	—	2–4	2–4	2–5	2–5	3–6	4–7	2–4	4–8

* *C. finmarchicus* and *C. helgolandicus* cannot be distinguished until Copepodite V.

The setules on the maxillae of all the copepodite stages and of both *C. finmarchicus* and *C. helgolandicus* were examined and measured to give some idea of the size of organism which could be retained (Fig. 2 and Table 1).

There are altogether twenty-nine setae, of which twenty form a sheet approximately in the same plane, the gaps between them being partly filled by closely set setules. Usually each seta bears two rows of setules set at an angle of about 90° to one another, sloping forward and making an angle of about 60° with the seta. All but one (the external) of the remaining setae are on the inner side of the limb, one to each endite except the fifth and one to each of the three terminal segments; they usually lie at an angle to the main setae. Those on endites 1–4 are shorter and have irregularly spaced setules projecting in all directions. That on the first endite is like the others on the upper half only; on its lower half there are two regular rows of setules at 180° in the plane of the setae. That on the terminal segment of the limb has two opposite rows of closely set setules (like a feather) but it is naked in the upper half. Finally, there is a short external seta on the coxa with opposite rows of very fine and closely set setules.

Apart from the setules most of the main setae are serrated towards the tip, the serrations sometimes enlarging to become a series of short spines between the setules.

It is not easy to understand the purpose of all this elaboration. Obviously the setules forming a close network between the setae must be the main instruments of filtration, and the fact that the setules and serrations are both directed forward will make it easy for a particle to pass towards the mouth. The function of the special internal setae and of the external seta is unknown. The whole limb is slightly concave viewed from the outer side.

With the exception of the first and sixth endites on which one or two setae are added during development, the main setae are present in all the copepodite stages and indeed almost all are present in Nauplius VI.

The maxillae were dissected off from several specimens of each stage and the distance apart of the setules measured on one or more setae of each endite of the coxa and basipod as well as on most of the segments of the endopod. The smallest divisions on the micrometers used measured 1·86 and 1·67 μ. It was not always possible to make measurements on the same part of the setae or even on the same setae on different individuals, so that the results which are shown in Table I indicate the variations only approximately.

It can be seen that the smallest distance between the setules lies between 2 and 3 μ. In general the setules are closest towards the lower half of the setae and widest towards the tip; one distance may be double the other. There is a gradual but less regular widening of the distance between setules from endite 1 of the coxa to segment 5 of the endopod. On the two terminal setae the spacing is much wider and may reach 20 μ. Although the minimum distance of 2–3 μ is found also in the adult (chiefly at the base of the coxal setae) there is a tendency for the setules to be closer in the younger stages.

There seems also to be a real difference between the adults of the *finmarchicus* and the *helgolandicus* forms; the setules are between 50 and 100% wider apart in the latter. This difference is less marked in Stage V. The distances apart are considerably smaller than Ussing's (1938) estimate. According to him the smallest distance apart of the setules in the adult female is 5·7 μ, in Stage V, 3·8 μ, and in Stage IV, 3·2 μ. His specimens were from the Arctic where the *Calanus* are much larger than ours and the size difference may account for the greater minimum distance apart.

It is not possible to say how efficient the maxilla of *Calanus* is as a filter but we should expect considerably smaller particles to be retained than have been shown experimentally to be important as food. The size of some of the cells used in the feeding experiments is shown in Fig. 2. It is obvious that the small flagellate *Nannochloris* can easily pass the filter over most of its area.

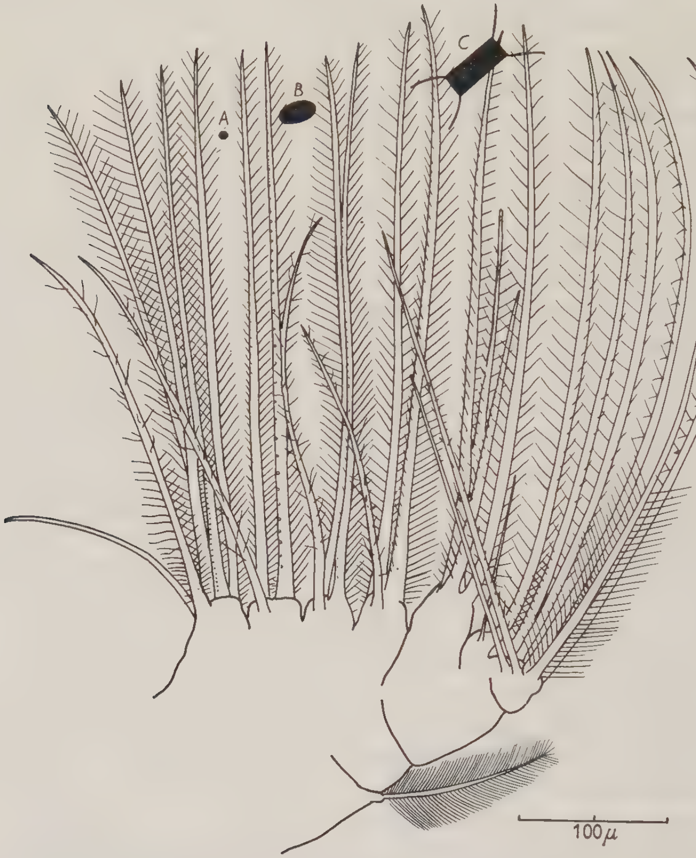

Fig. 2. Left maxilla of *Calanus helgolandicus* ♀ from the right. *A*, *B* and *C* represent the sizes of food organisms used. *A*, *Nannochloris oculata*; *B*, *Syracosphaera elongata*; *C*, *Chaetoceros decipiens* (from culture).

FEEDING EXPERIMENTS

One certain test of whether a nauplius can eat any particular food is to find the skeleton in the faecal pellets. This is sometimes very difficult if the skeleton has no obvious sculpturing. It is probable, for instance, that Nauplius III can eat *Chaetoceros decipiens* but the frustule of this diatom is thin-walled and has no striking features and it has not been identified in faecal pellets. *Skeletonema* frustules are found complete in the faecal pellets of Nauplius III showing that this common spring diatom is a food for even the earliest feeding stages of *Calanus*. Coccoliths are shed in large numbers when a culture of

Syracosphaera is getting old, and it is possible that some may be taken loose. However, the faecal pellets examined from nauplii which had been feeding on a culture of this species were packed full of coccoliths, which had probably come from ingested cells.

These and other observations for the later nauplius stages are shown in Table II and the size of some of the faecal pellets given. It shows a gradual increase with the size of the nauplius, as might be expected. The pellets were usually ovoidal but were very pale compared with those from adults on the same food. The rate of production with a suitable food is somewhat similar to that of the adult *Calanus*.

TABLE II. SIZE OF FAECAL PELLETS IN NAUPLIUS STAGES

(In those marked with an asterisk, the food organism was identified in the faecal pellet.)

Food organism	Size of food organism (μ)	Size of faecal pellets (μ)			
		N III	N IV	N V	N VI
Skeletonema costatum	4–5	48 × 24*	46 × 25*	83 × 33*	87 × 30*
Chaetoceros decipiens	14–21 × 20–78	33 × 20	—	—	—
Syracosphaera elongata	18–30 × 12	31 × 17*	39 × 21*	48 × 28*	*
Prorocentrum micans	43 × 27	—	—	60 × 29*	87 × 32*
P. triestinum	10 × 14	*	—	—	*
Peridinium trochoideum	25 × 19	—	—	—	*
Ditylum brightwellii	20–60 diam.	—	—	—	*

The earlier the stage of the *Calanus* the more difficult are the faecal pellets to recognize. When Janus green is added to the water, they take up the stain and are slightly easier to see, but the risk of losing some is much greater with nauplius stages than with copepodites. In the quantitative experiments in which the faecal pellets were picked out, the percentage of the phosphorus-containing portion digested was always high and it was felt that little was gained by a time-consuming and perhaps unsuccessful attempt to find all the faecal pellets produced. In many of the experiments therefore faecal pellets were ignored and a larger number of individuals could thus be tested. The total variation was so great that in comparing volume filtered with faecal pellets included and excluded, the first does not show a consistently higher figure; the reading of the body alone is therefore justifiable.

Grobben (1881) has stated that the first nauplius stage has no anal opening and this has been confirmed by Miss Judith C. Perryman of King's College, London (personal communication). She finds that the mouth does not open until Nauplius II and the anus not until Nauplius III. From our experiments also it seems certain that neither Nauplius I nor Nauplius II feeds. When Nauplius II were used some of them always moulted during the course of the experiment, and from the results shown in Table III it is clear that only those nauplii which reached Stage III had taken up an appreciable amount of food. The slight radioactivity shown by the Nauplius II may be due to ^{32}P absorbed from solution.

The results of twenty-two out of the thirty-nine experiments done on the late nauplius and early copepodite stages are shown in Table IV. The rest are omitted for one reason or another. In nine the copepods had eaten badly; it was considered that if Nauplius III had filtered less than an average of 0·05 ml. in 24 h they must have been unhealthy. If, however, two food species were tested in the same experiment and the copepods did well in one and badly in the other, both results were accepted. Four experiments on *Nannochloris* and *Monochrysis* are excluded; these are very small flagellates, not likely to be taken freely, but no check against a good food was made. Two experiments on Nauplius II (which does not feed) and two on *Bacillus globigii*, which gave negative results, are also omitted.

TABLE III. UPTAKE OF ^{32}P IN LATE N II AND EARLY N III

(Duration of experiments between 18 and 24 h)

Culture	Age of culture (days)	Concentration cells/ml.	Nauplii put in	Nauplii as read	Counts/ min	Counts/ nauplius/ min
Skeletonema	7	275	20N II	2N II	112	56
(contaminated)				17N III	7096	417
26. viii. 54			20N II	5N II	90	18
				14N III	4405	315
Chaetoceros	6	1670	20N II	2N II	122	61
decipiens				9N III	10146	1127
21. iv. 55				8N III	8134	1017
			23N II	5N II	78	16
				12N III	11133	928
			20N II	4N II	19	5
				8N III	9139	1142
				9N III	5649	628
Ditylum						
brightwellii	18	57	20N II	5N II	241	48
6. vi. 55				12N III	1176	98
			20N II	7N II	177	25
				10N III	5567	557
Dicrateria						
inornata	6	71000	20N II	1N II	5	5
21. iv. 55				13N III	5218	401
26. viii. 54	7	43000	20N II	5N II	1	0
				13N III	4180	322
			20N II	9N II	27	3
				9N III	2910	323

In any one experiment also the result was rejected for any individual which had done very much worse than its fellows. Sometimes these were noted as being inactive or unhealthy, but sometimes they were apparently in as good condition as the rest. They may, however, have been near moult (see Tables V and VII). The results given in the table are therefore selected, but as explained above it is thought that they probably show the normal behaviour.

On one or two occasions Nauplii III and IV reared in the laboratory were separated into *Calanus finmarchicus* and *C. helgolandicus* and read separately. The former is always smaller and gave, as might be expected, lower results.

TABLE IV. FEEDING EXPERIMENTS ON NAUPLII AND COPEPODITES

Number of copepods used in each experiment and volume filtered in 24 h

Food species and size	Date	Age of culture (days)	Culture cells/ml.	NIII		NIV		NV		NVI		CI		CII		CIII	
				No. used	Average ml./24 h	No. used	Average ml./24 h	No. used	Average ml./24 h	No. used	Average ml./24 h	No. used	Average ml./24 h	No. used	Average ml./24 h	No. used	Average ml./24h
Skeletonema costatum, 4–5 μ	9. vi. 54	7	10,200	—	—	—	—	—	—	—	—	5	0·53	—	—	—	—
	29. iii. 55	6	9,440	29	0·08	—	—	3	0·26	14	0·30	2	0·40*	4	2·04	2	3·49
	4. iv. 55	12	1,290	61	0·07	—	—	—	—	—	—	—	—	—	—	—	—
	26. viii. 54	7	275†	8	0·24	—	—	2	0·72	3	0·72	5	0·92	—	—	—	—
Chaetoceros decipiens, 14–21 μ diam., 20–78 μ long	17. viii. 54	19	4,090	—	—	—	—	1	0·12*	—	—	—	—	—	—	—	—
	4. x. 54	8	68	49	0·28	15	0·21	—	—	—	—	—	—	—	—	—	—
	19. iv. 55	4	2,260	—	—	—	—	—	—	1	0·54*	2	0·51*	7	1·87	7	1·94
	19. iv. 55	4	2,260	—	—	—	—	—	—	—	—	1	0·77	—	—	—	—
	21. iv. 55	6	1,670	33	0·05	6	0·22	5	0·32	1	0·48	1	0·60	2	0·51*	—	—
	26. iv. 55	11	1,480	—	—	—	—	1	0·31*	1	0·47*	—	—	—	—	—	—
	26. iv. 55	11	1,480	—	—	—	—	1	0·23	1	0·23	—	—	—	—	—	—
	27. vi. 55	4	315	17	0·09	3	0·66	2	0·78	5	1·04	2	1·44	9	3·19	6	5·67
Ditylum brightwellii, 20–60 μ diam.	6. vi. 55	18	57	38	0·05	7	0·14	7	0·15	3	0·35*	9	0·61	3	0·83*	—	—
	6. vi. 55	18	57	—	—	—	—	—	—	9	0·37	—	—	5	1·07	—	—
Nannochloris oculata, 2–4 μ	26. viii. 54	17	32,000	15	0·03	5	0·07	5	0·04	6	0·05	3	0·07	7	0·27	—	—
	4. iv. 55	12	19,600	59	0·01	1	0·01	4	0·00	4	0·00	—	—	3	0·11	2	0·07
Dicrateria inornata, 3–5·5 μ	23. viii. 54	4	130,000	51*f*	0·04	7	0·05	—	—	—	—	18	0·14	—	—	—	—
	23. viii. 54	4	130,000	35*h*	0·05	—	—	—	—	—	—	—	—	—	—	—	—
	19. iv. 55	4	112,000	—	—	—	—	—	—	—	—	2	0·56*	3	1·83	4	1·28
	19. iv. 55	4	112,000	—	—	—	—	—	—	—	—	3	0·44	—	—	—	—
	21. iv. 55	6	71,000	38	0·07	1	0·12	4	0·15	1	0·07*	—	—	—	—	—	—
	21. iv. 55	6	71,000	—	—	—	—	—	—	4	0·04	—	—	—	—	—	—
Syracosphaera elongata, 18–30 × 12 μ	9. viii. 54	11	1,250	1	0·04*	1	0·04*	1	0·44*	1	0·67*	—	—	1	0·80*	—	—
	9. viii. 54	11	1,250	9*f*	0·11	3*f*	0·17	—	—	—	—	—	—	—	—	—	—
	9. viii. 54	11	1,250	9*h*	0·25	2*h*	0·25	—	—	—	—	—	—	—	—	—	—
	11. viii. 54	13	570	6	0·08	1	0·41*	3	0·59*	1	0·22*	2	1·08	—	—	—	—
	28. iv. 55	13	64	24	0·08	—	—	—	—	—	—	—	—	—	—	—	—
	9. v. 55	24	370	20	0·05	3	0·06	4	0·10	10	0·12	—	—	—	—	—	—
	27. vi. 55	4	320	26	0·17	3	0·75	11	1·01	11	1·36	7	2·12	13	2·95	14	4·45
Prorocentrum triestinum 10–14 μ	8. vi. 55	22	1,515	21	0·01	5	0·05	10	0·07	6	0·10	9	0·18	8	0·30	—	—

* Faecal pellets included. *h* = *helgolandicus*, *f* = *finmarchicus*. † contaminated with flagellates.

TABLE V. UTILIZATION OF *SYRACOSPHAERA ELONGATA*

(Culture 4 days old. 14·5° C. 27–28 June 1955)

			Calanus				ml. filtered in 24 h	
Concentrations	Vol. of bottle ml.	Time (h)	Put in	As read	Counts/min	Total	Total	/*Calanus*
5120 c/ml./min 320 cells/ml.	34·86	24¼	21 N III	8 N III	6,613	14,288	2·89	0·17
				8 N III	7,384			0·19
				1 N IV	291			0·02
	35·28	24	20 N III	10 N III	7,330	10,854	2·20	0·15
				9 N III*	3,524			0·08
	37·50	16	4 N IV*	1 N IV	3,953	6,233	1·84	1·17
				1 N IV–V	195			0·06
				1 N IV–V	447			0·13
				1 N V	1,638			0·48
	37·50	24	4 N IV	1 N IV	2,283	5,501	1·09	0·45
				1 N IV	3,147			0·62
				1 N IV–V	71			0·01
	69·54	22½	10 N V	1 N V	4,068	27,524	5·96	0·88
				1 N V	4,746			1·03
				1 N V	4,142			0·90
				1 N V	4,706			1·02
				1 N V–VI	273			0·06
				1 N V–VI	1,704			0·37
				1 N V–VI	1,202			0·26
				1 N V–VI	404			0·09
				1 N VI	2,025			0·44
				1 N VI	4,254			0·92
	70·68	19¼	10 N V	1 N V	6,294	43,835	11·39	1·64
				1 N V	3,044			0·79
				1 N V	1,607			0·42
				1 N V	5,286			1·37
				1 N V	4,867			1·27
				1 N V	6,587			1·71
				1 N V	3,346			0·87
				1 N V–VI	3,623			0·94
				1 N V–VI	5,929			1·54
				1 N V–VI	3,252			0·85
	70·26	21½	10 N VI	1 N VI	6,803	65,128	15·68	1·64
				1 N VI	1,708			0·41
				1 N VI	5,063			1·22
				1 N VI	4,647			1·12
				1 N VI	8,254			1·99
				1 N VI–C I	7,558			1·82
				1 N VI–C I	6,213			1·50
				1 N VI–C I	10,570			2·55
				1 C I	6,798			1·64
				1 C I	7,514			1·81
	70·93	22¼	10 N VI	1 N VI	6,894	53,658	12·23	1·57
				1 N VI	6,207			1·41
				1 N VI	6,938			1·58
				1 N VI	4,703			1·07
				1 N VI	6,263			1·43
				1 N VI*	6,690			1·52
				1 N VI–C I	5,529			1·26
				1 N VI–C I	4,155			0·95
				1 N VI–C I	4,020			0·92
				1 N VI–C I	2,259			0·52

* Sluggish.

TABLE V (*continued*)

			Calanus				ml. filtered in 24 h	
Concentrations	Vol. of bottle ml.	Time (h)	Put in	As read	Counts/min	Total	Total	/*Calanus*
5120 c/ml./min 320 cells/ml.	72·79	21¾	4C I	1C I	8,107	39,577	9·02	1·85
				1C I	10,064			2·29
				1C I	12,307			2·81
				1C I	9,105			2·08
	74·70	21¾	5C I	1C I	8,225	35,922	8·14	1·87
				1C I	10,079			2·28
				1C I	7,300			1·66
				1C I*	2,522			0·57
				1C I–C II	7,796			1·77
	66·20	18¼	10C II	1C II	7,818	93,579	28·12	2·35
				1C II	9,662			2·90
				1C II	14,305			4·30
				1C II	2,476			0·74
				1C II	13,967			4·20
				1C II	21,461			6·45
				1C II	12,210			3·67
				1C II–C III	11,680			3·51
	68·72	22½	10C II	1C II	15,751	74,031	17·30	3·68
				1C II	8,570			2·00
				1C II	671			0·16
				1C II	9,292			2·17
				1C II	10,004			2·34
				1C II	14,403			3·37
				1C II–III	282			0·07
				1C II–III	3,940			0·92
				1C III	11,118			2·60
	75·73	15¾	12C III	1C III	4,304	127,284	45·91	1·55
				1C III	13,802			4·98
				1C III	11,032			3·98
				1C III	21,817			7·87
				1C III	11,322			4·09
				1C III	17,568			6·34
				1C III	15,765			5·69
				1C III	25,540			9·21
				1C III*	3,866			1·39
				1C III–IV	2,268			0·82
	76·76	21¾	12C III	1C III	6,990	92,284	22·68	1·72
				1C III	5,744			1·41
				1C III	16,078			3·95
				1C III	5,837			1·43
				1C III	7,912			1·95
				1C III	5			0·00
				1C III	33,109			8·14
				1C III*	4,490			1·10
				1C III*	5,549			1·36
				1C III–IV	6,570			1·61
Filtrate 35 counts/ml./min	32·38	23	5N III	4N III	0	—	—	—
			1N V	1N V	0	—	—	—
			2N VI	2N VI	0	—	—	—
			2C II	1C II	2	—	—	—
			2C III	1C II	0	—	—	—
				1C III	3	—	—	—
				1C III	2	—	—	—

* Sluggish.

As Table IV shows, the volumes filtered vary much even with the same species of food organisms. On the whole, as is natural, the volume filtered rises from one stage to the next. The very small flagellate *Nannochloris* is clearly used no better by the nauplii and early copepodite stages than it is by the adult. Thus in two experiments the utilization of *Nannochloris* was compared with that of *Skeletonema*. In the first a fairly long range of stages was compared in *Nannochloris* and in a contaminated *Skeletonema* culture. In every one the volume filtered in *Nannochloris* culture was much smaller. In the second a long range of stages was tested in *Nannochloris* and this was checked only by Nauplius III in *Skeletonema*. Both experiments showed that the low results in *Nannochloris* were not caused by unhealthy copepods.

To test whether really small particles could be filtered off, an experiment was made with radioactive bacterial spores (*Bacillus globigii*, 0·7 μ in volume). But, as with adults, none was retained.

It is surprising to find that a large diatom such as *Ditylum* is apparently taken up by even the small nauplii, but possibly debris or broken cells, some of which are always found in old cultures, are being ingested.

The maximum filtration by Nauplius III found in any experiment was 1·0 ml. in 24 h; by Nauplius IV, 1·2 ml.; by Nauplius V, 1·7 ml.; by Nauplius VI, 2·0 ml.; by Copepodite I, 2·8 ml.; by Copepodite II, 6·4 ml.; and by Copepodite III, 9·2 ml. Most of these maxima were reached in one experiment comparing the utilization of *Chaetoceros decipiens* and *Syracosphaera elongata*. Details of the second may be seen in Table V. This experiment, done at the end of June, gave quite exceptionally high figures for all the stages tested. The results also show the great variation which may occur even among copepods of the same stage.

In this experiment, as in others, there were many moults. As a rule animals found in the process of moulting gave a low reading, whereas those which had successfully moulted might be as high as the rest, but this was not invariable. Although several individuals were used in each bottle, the total volume filtered was not a high proportion of its contents except with Copepodite III. In this case the animals may have been too crowded and might have filtered more had they been put singly in similar bottles.

Not all the *Calanus* put in were recovered. Some nauplii may die and disintegrate during an experiment, and occasionally one or two individuals are lost when the stopper of the bottle is put in.

Fig. 3 shows the average results obtained in all the experiments of Table IV for a number of different food species. There appears to be no abrupt change in the volume filtered with the change from nauplius to copepodite, indeed the change from the first to the second copepodite is usually more marked.

The results of three experiments in which faecal pellets were collected are shown in Table VI. That on *Skeletonema costatum* indicates that the percentage of ^{32}P retained is considerably lower than with the other two species.

This comparatively low assimilation of *Skeletonema* was found also with adults. In the experiments on *Ditylum brightwellii* the Nauplius VI have retained a larger proportion than the Copepodite II. This may be partly because not all the faecal pellets were found; even in the unlikely event of a 50% loss, however, the digestion would still be higher than in Copepodite II.

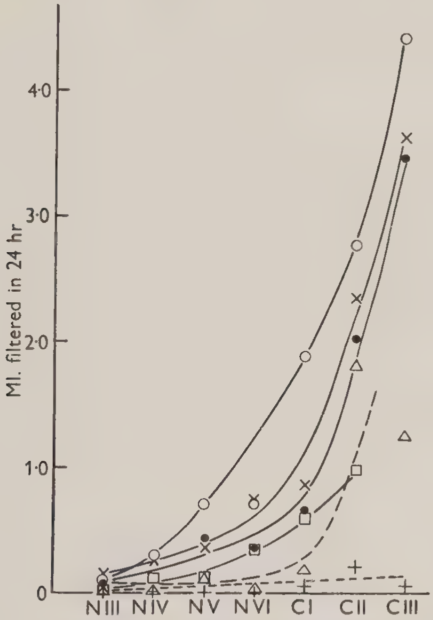

Fig. 3. Average volumes filtered in 24 h by stages Nauplius III to Copepodite III in various food species. ○, *Syracosphaera*; ×, *Chaetoceros*; ●, *Skeletonema*; □, *Ditylum*; △, *Dicrateria*; +, *Nannochloris*.

TABLE VI. UTILIZATION, INCLUDING FAECAL PELLETS, OF THREE FOOD ORGANISMS

Concentration	Age of culture (days)	Date	Time in h	Stage of *Calanus*	Faecal pellets No.	Faecal pellets Counts/min	Body less filtrate	Total removed, c/min	Percentage used	ml. filtered in 24 h
Skeletonema 91,125 c/ml./min 266,000 cells/ml.	7	9. vi. 54	18	CI	11	72	772	844	91·5	0·39
				CI	40	355	569	924	61·6	0·43
				CI	30	293	895	1,188	75·3	0·56
				CI	35	562	538	1,100	48·9	0·51
				CI	57	531	1,092	1,623	67·3	0·78
Ditylum 36,610 c/ml./min, 57 cells/ml.	18	6. vi. 55	20·5	NVI	16	558	10,536	11,094	95·1	0·38
				NVI	18	612	8,529	9,141	93·3	0·31
				NVI	13	425	9,886	10,311	95·9	0·35
				CII	48	5339	24,270	29,609	82·0	1·10
				CII	43	4210	14,733	18,943	77·8	0·68
				CII	33	3396	16,095	19,491	82·6	0·72
Syracosphaera 9725 c/ml./min 1250 cells/ml.	11	9. viii. 54	16	NIII	1	3	228	231	98·7	0·04
				NIV	7	8	2,351	2,359	99·7	0·38
				NV	9	9	2,644	2,653	99·7	0·44
				NVI	26	76	3,873	3,949	98·1	0·67
				CII	15	86	4,442	4,528	98·1	0·80

In the experiment with *Syracosphaera elongata* the faecal pellets of the smaller nauplii were probably not all found. The Nauplius III filtered only a small volume and was probably not in good condition.

In general the proportion assimilated of the phosphorus-containing part of the different food species used varies as it does in adults. *Syracosphaera* and *Chaetoceros* show a high figure (over 90%), *Ditylum*, *Skeletonema* and the small flagellate *Dicrateria* a rather lower figure.

DISCUSSION

The experiments have shown several points of interest in the feeding of the early stages of *Calanus*. The failure of Nauplius I to feed was expected because its gut has no anal opening, but the failure of Nauplius II was unexpected until examination revealed that it had no anal opening either. This, however, explains why it is easy to rear *Calanus* in the laboratory from egg to Nauplius III, the first feeding stage. After this mortality is high. In the experimental *Calanus* taken from tow-nettings and used the same day, moulting, as is shown in Table V, is not always successful. Thus the experimental *Calanus* contain a high proportion of individuals unlikely to survive and this shows in a failure to feed or in a failure to moult successfully.

TABLE VII. FREQUENCY OF MOULT IN EXPERIMENTS

Stage	Total no. used	No. moulting	Percentage moulting
NIV	85	30	35·3
NV	161	66	41·0
NVI	234	91	38·9
CI	155	28	18·1
CII	92	21	22·8
CIII	84	12	14·3

Table VII shows the numbers of *Calanus* used in all the experiments made between April and August and the percentage which reached moult. Since the experiments lasted less than 24 h it may be deduced that *Calanus* remains as Nauplii IV, V and VI only 2–3 days each, as Copepodites I and II about 5 days each and as Copepodite III about 7 days. Since Nauplius III was used as a rule immediately after its moult from Nauplius II, nothing can be deduced about the duration of this stage. The times agree fairly well with those given by Nicholls (Marshall & Orr, 1955*a*, p. 78) but are much less than the times suggested for the North Sea by Rees (1949) and Cushing (1955).

One result of these experiments on the young stages of *Calanus* is to throw doubt on the assumption that the earlier the stage the smaller will be the food organism which it requires. Although from a study of the setulation it seems that even an adult *Calanus* should be able to retain particles of 2–3 μ by at least some part of its filtering apparatus, in practice it hardly does so at all. In the nauplii the distance between setules is not much smaller than in the adult, the filtering screen looks less efficient, and in fact the nauplius seems to feed on much the same as the adult except for really large cells. The filtering apparatus has not a rigid mesh and it would be easy for particles which could be retained by the closest setules to slip through where they are wider, or to escape between the setae.

Cushing (1955) has seen a *Pseudocalanus* take a large *Biddulphia* cell, break it and filter off some of the contents. If *Calanus* nauplii can also break diatoms and remove the contents without ingesting the cell, they may be able to feed on even the largest species.

Table VIII. Maximum Uptake of Plant Cells by one *Calanus* in 24 h in Sea-Water Concentrations*

Species	No. cells/ml.	Counts/ cell	N III		N IV		N V		N VI	
			Cells taken in	ml. filtered	Cells taken in	ml. filtered	Cells taken in	ml. filtered	Cells taken in	ml. filtered
keletonema costatum	10,200	1·5	—	—	—	—	—	—	—	—
	9,440	0·31	2845	0·32	—	—	2539	0·28	4515	0·51
	1,290	5·7	77	0·09$_{18}$	—	—	—	—	—	—
haetoceros decipiens	68	87·0	64	0·98	—	—	—	—	—	—
	1		2	0·34$_{11}$	—	—	—	—	—	—
	2,260	22·4	—	—	—	—	—	—	—	—
	1,670	27·8	98	0·06$_{7}$	479	0·29	847	0·52	733	0·48
	1,480	29·0	—	—	—	—	448	0·31	652	0·47
	315	125·0	30	0·10$_{10}$	211	0·69	251	0·82	385	1·29
itylum brightwellii	57	642·0	3	0·05$_{10}$	12	0·20$_{2}$	15	0·27	31	0·56
yracosphaera elongata	64	38·0	71	0·11$_{10}$	—	—	—	—	—	—
	320	16·0	57	0·19$_{8}$	371	1·17	513	1·71	576	1·99

Species	No. cells/ml.	Counts/ cell	C I		C II		C III	
			Cells taken in	ml. filtered	Cells taken in	ml. filtered	Cells taken in	ml. filtered
keletonema costatum	10,200	1·5	6979	0·78	—	—	—	—
	9,440	0·31	5454	0·65	23,800	2·72	35,140	3·90
	1,290	5·7	—	—	—	—	—	—
haetoceros decipiens	68	87·0	—	—	—	—	—	—
	1		—	—	—	—	—	—
	2,260	22·4	1602	0·78	5,339	2·77	5,632	3·06
	1,670	27·8	991	0·60	—	—	—	—
	1,480	29·0	—	—	—	—	—	—
	315	125·0	481	1·56	1,272	4·39	2,353	8·24
tylum brightwellii	57	642·0	53	0·96	74	1·35	131	2·44
yracosphaera elongata	320	16·0	849	2·81	1,764	6·45	2,283	8·14

*When more than one copepod was used at one time (N III and N IV) the number is shown as a suffix.

The data in Fig. 3 and Table IV (p. 595) show that the average volume filtered in 24 h by healthy individuals varies from about 0·1 ml. for Nauplius III up to between 2 and 4 ml. for Copepodite III. The rate increases rapidly with development but the variation is great. Compared with adults and Stage V copepodites the volumes filtered are relatively high.

The results can also be expressed as cell equivalents. Table VIII shows the maximum intake of cells in experiments where the culture concentration was such as might be found in the sea, although the numbers in the first two *Skeletonema* cultures would be found only during a rich diatom increase. A single Nauplius III took in *Skeletonema* cells at the rate of about two a minute and in the same experiment Copepodite III took them up at the rate of twenty-five a minute. Remains of *Ditylum* cells were not recognized in any stage below Nauplius VI so the figures for the earlier nauplii probably mean little.

No data on respiration are available so that the volumes filtered cannot be related to food requirements nor these to the food available in the sea.

We are very grateful to Dr Mary Parke and Miss D. Ballantine of the Plymouth Laboratory and to Dr M. R. Droop of this laboratory for providing us with cultures. The radioactive bacterial spores were kindly supplied by Dr D. W. Henderson of the Experimental Establishment, Porton. We are also grateful to Dr Richard B. Pike for help with Fig. 2. We should like to thank Miss E. R. Wallace for help with the radioactive counts.

Summary

Nauplii and early copepodites of *Calanus* were fed on radioactive cultures of diatoms and phytoflagellates and the food uptake and volumes filtered were measured.

Nauplius Stages I and II do not feed; Nauplius III can filter up to a maximum of 1 ml. in 24 h. There is an increase of volume filtered with development up to a maximum of about 9 ml. in 24 h for Copepodite III.

Organisms of up to 20 μ (*Skeletonema costatum*, *Prorocentrum triestinum*, *Syracosphaera elongata*) can be eaten by Nauplius III but, as with adults, the very small flagellate *Nannochloris oculata* (2–4 μ) is not taken in to any extent by the young stages.

The filtering appendages are described. The minimum distance between the setules is about 2 μ but for the main filtering surface it varies from 2 to 11 μ, and shows little variation with stage.

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AMALOSOMA EDDYSTONENSE SP.N., A NEW SPECIES OF BONELLIIDAE

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In October 1921, a specimen of an echiuroid was taken in the otter trawl of the S.S. *Salpa*, in the neighbourhood of the Eddystone lighthouse. The specimen, a female, was provisionally identified by Mr E. Ford as *Hamingia arctica* Kör & Dan. The pharynx and uteri were opened in an unsuccessful search for males, after which the specimen was preserved in alcohol and put in the museum at the Plymouth Laboratory. In the following month, two more specimens were taken in the dredge by Dr R. S. Clark, a few miles from the same locality, and placed in the museum without further examination. No more specimens were obtained until April 1925, when one was taken in a trawl off Rame Head, near the entrance to Plymouth Harbour. This specimen was examined by O. D. Hunt, and was found to be a female with uteri containing ripe eggs. Twenty-eight males of minute size were discovered attached externally to the female in a groove in the skin which extended for a short distance in the median line just posterior to what appeared to be a single genital orifice.

The males described by Lanchester from the pharynx of *Hamingia arctica*, and those from the uterus of *Acanthohamingia* described by Ikeda, closely resemble those of *Bonellia* in possessing a ciliated epidermis and in their nematoform shape. The males found by Hunt, however, were neither ciliated nor of such an elongated form. This difference showed at once that the species was not *Hamingia arctica* as supposed. Hunt therefore re-examined all the specimens in the Museum, and in each case males of the new type were found attached externally in a similar fashion and in a similar position.

Partially embedded in the thick epidermis of the female, these males are easily overlooked, as they had been on the specimen examined by Ford, on which Hunt subsequently found a single male. The two other specimens provided thirty-eight and thirty males respectively.

Four more specimens have been obtained since then from the same locality, two with attached males and two with none.

Since it was decided to include the records of this capture in the new edition of the *Plymouth Marine Fauna*, it finally became necessary to name it. Hunt, in manuscript 1925, recognized that the animals belonged to a new genus and species, but owing to pressure of work was unable to complete his observations. To him I am indebted for his generous gesture in placing his notes, drawings and specimens at my disposal, and to Dr D. P. Wilson for the suggestion that I should have the privilege of examining and reporting on the material.

CLASSIFICATION OF THE BONELLIIDAE

The classification of the Bonelliidae has always presented difficulties, and several attempts have been made to clarify it. A revision undertaken by Fisher summarized the state of the accepted classification (1946, pp. 249–62) and he described three new genera. New material, and the fact that he had overlooked an important paper by Monro (1927), caused him to study the family again, and in the following year (Fisher, 1947), he published a fresh revision, adding three more new genera, including the genus *Amalosoma*, which interests us here. Most of his new genera contain but a single species, and it may be felt that more genera have been created than necessary, but at least he has put the classification on a reasonable basis. I have not had the opportunity of examining many animals belonging to this family and so have no personal comments to make on his classification, which I have adopted.

GENUS *AMALOSOMA* FISHER, 1947

Fisher (1946, pp. 260–2, pls. 31, 32) described a new species of *Acanthohamingia* Ikeda, from Japan, which he named *A. paradola*, and which differed in several important particulars from the two previously described species of this genus, *A. shiplei* and *A. ijimai*. In his second revision, he recognized that these differences were sufficient to warrant the creation of a new genus which he called *Amalosoma* (Fisher, 1947, p. 856), the generic criteria being the absence of minute setae in the genital groove of the female; the anal trees being numerous, slender, sparsely branched, arising for the most part independently from the very thin wall of the cloaca; there being two nephridia; and the male possessing a pair of curved setae.

A second species, so far known only as occurring in the Plymouth area, is now added to the genus.

Amalosoma eddystonense n.sp.

Holotype—female

The body is 144 mm in length, and no proboscis is preserved. Judging, however, by the appearance of the anterior portion there has been one, but probably broken off and lost during dredging. The body wall on the anterior and posterior thirds is solid, cylindrical and heavily papillated, and the diameters of these two portions are some 13 and 15 mm respectively. The middle third is swollen to the appearance of a thin-walled balloon, some 25 mm in diameter, through which the viscera can be seen.

The skin is covered, except in the thin portion, with numerous irregularly shaped flattened papillae, which are most numerous and largest anteriorly and posteriorly, less numerous in the middle portions of the body, and smallest ventrally.

The internal anatomy, so far as can be made out in its slightly macerated state, closely resembles that of *A. paradola*. The pharynx is similarly enlarged, and the arrangement of the neuro-intestinal blood vessels, so far as can be seen, is much the same. The gut is also long, as in the previous species. In *A. paradola*, Fisher described the nerve cord as being extremely thin, only 0·135 mm in diameter, but in this species it seems normal and the average diameter is 0·5 mm.

The two nephridiophores are packed with large ova, which have an average diameter of 0·45–0·5 mm. The ova are not enclosed in a follicle.

The anal trees, in all specimens, are much macerated, but arise as single stems from the cloaca, as described for *A. paradola*. They are, however, fewer, larger and more branched than in that species, to judge by Fisher's figure (1946, pl. 31, fig. 6). In the specimen dissected and drawn by Hunt, but not included in the material I have seen, they seem to have filled the posterior third of the body with a solid mass of tubules. Each branch ends in a conical-shaped wide-mouthed cup.

Male

The male is small, usually just under 2 mm in length, planiform or oblong. It is attached externally to the female in the nuptial groove by two hooked genital setae, which have comparatively large and well-developed muscular attachments. The body wall has two muscle layers, circular and longitudinal. The cuticle is not ciliated.

It agrees in most respects with Fisher's description of *A. paradola*.

The other females are very much macerated internally. Most are cylindrical in shape, but one resembles the type in having a thin expanded middle portion. In these the papillae are largest and most numerous anteriorly and posteriorly, less numerous in the middle region and least numerous ventrally. The colour of the spirit specimens is a uniform greyish yellow; in life these animals are a dull mid-green on the papillae with a similar but paler colour between them.

The species is referable to the genus *Amalosoma*, and there is not a great deal of difference between *A. paradola* and *A. eddystonense* but I hesitate to consider them as belonging to a single species. The chief differences are shown in Table 1.

Distribution. Eddystone grounds Oct. and Nov. 1921, Sept. 1926, May 1927. (Marine Biological Association, 1931, p. 148). Eddystone grounds and Rame Head, April 1925, July 1929 (specimen submitted by O. D. Hunt).

Breeding. Of the specimens examined only the type carried ova. This specimen, taken in April 1925, has the nephridia occupying about one-eighth of the body cavity; full of ova.

TABLE I

	A. paradola	*A. eddystonense*
Body	Pear-shaped	Sausage-shaped
Body wall	Very thin, viscera visible	Body-wall very thick: in only two specimens is there a thin-walled median enlargement
Nerve cord	Very thin, 0·135 mm	Thick, 0·5 mm diameter
Anal trees	Arising, one presumes, as numerous individual stems from the cloaca; branches bearing funnels few, short, not branching. Funnels vase-like, widest at the base	Arising from a few individual stems from the cloaca; branches bearing funnels numerous, long and sometimes themselves branching. Funnels ciliated, conical, widest at the mouth

SUMMARY

The animals recorded as *Hamingia arctica* Kören & Danielssen in the *Plymouth Marine Fauna* (Marine Biological Association, 1931) were recognized some years ago by O. D. Hunt as belonging to a, then, new genus and species. His description was, unfortunately, never published, and his genus must be suppressed in favour of *Amalosoma* created by Fisher in 1947. His species remains valid but the name he originally suggested, *eddystonia*, from the locality in which it was first taken, has been changed to *eddystonense* in accordance with accepted practice.

A. eddystonense differs from the other described member of the genus, namely *A. paradola*, in having a cylindrical instead of a pear-shaped body; generally a thick cuticle; a thick instead of a thin nerve cord, and strong anal trees ending in conical cups.

The males, minute in size and deeply embedded in the thick epidermis of the genital groove of the female, are easily overlooked.

The species has been taken on several occasions near the Eddystone Lighthouse and off Rame Head, near Plymouth.

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A PRELIMINARY INVESTIGATION OF THE VARIATION OF VITAMIN B_{12} IN OCEANIC AND COASTAL WATERS

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(Text-figs. 1–4)

The presence in the sea of vitamin B_{12}, or of its analogues, has been found necessary for the growth of several species of unicellular plants (e.g. Provasoli & Pintner, 1953; Droop, 1954, 1955).

Bioassays of the quantities in inshore waters during winter have been made by Lewin (1954) and by Droop (1954). The present investigation, carried out in 1955, was designed to extend this study and compare different seasons of the year.

EXPERIMENTAL

In the work reported here the vitamin B_{12} content of oceanic and coastal waters in areas investigated regularly by vessels of the Scottish Home Department laboratory at Aberdeen was examined over a period of several months. Surface sea water and plankton samples were collected from the Butt of Lewis (58° 40′ N., 6° 10′ W.), the Norwegian Deeps (60–61° N., 3–4° E.), the Northern North Sea (58–60° N., 0–2° W.), the Faroe Channel (60° N., 7° W.) and the North Atlantic (58° N., 17° W.) during 1955. These areas contrast markedly in their content of planktonic indicator organisms (see for example, Fraser, 1952), and it seemed feasible that their biological differences might be causally related to differences in their content of micronutrients, including vitamin B_{12}. Thus it was hoped that if spatial and temporal differences in the vitamin B_{12} content of sea water occur, both would become evident during the course of the work.

In general three 4 l. samples were collected at each station and treated as follows: (*a*) filtered and preserved, (*b*) unfiltered and unpreserved (stored in darkness at 15° C), and (*c*) unfiltered and preserved. In addition, when circumstances permitted, samples were Seitz-filtered, thus removing any bacteria present, and preserved. The preservative used was that recommended by Hutner & Bjerknes (1948), consisting of a mixture by volume of 1 part *o*-fluorotoluene, 2 parts *n*-butyl chloride, and 1 part 1:2-dichloroethane. Plankton hauls were made at each station with vertically towed Hensen and Standard non-closing nets.

The Hensen hauls were made with a 70 cm diameter net of 60 meshes to the inch, from 100 m (or near the bottom in shallower water) to the surface. The Standard hauls were made with a 50 cm diameter net of 180 meshes per inch, towed from 50 m to the surface.

Preliminary experiments indicated that none of the organisms that can conveniently be used for assaying vitamin B_{12} grew in normal sea water, probably because of the salinity and the low concentration of the vitamin. Hence it was necessary to reduce the concentration of salt in the sea water and to concentrate the vitamin.

After experimenting with several approaches the following standardized procedure was adopted:

The pH of a 4 l. sample of sea water was adjusted to 5 by the addition of 8 g of potassium dihydrogen phosphate, 0·2 ml. of a 1 % solution of sodium cyanide were added and the mixture was maintained at 80–90° C for 20 min. After cooling the solution was saturated with liquid phenol (about 230 ml.) and the whole extracted with 10 successive 30 ml. portions of liquid phenol.

The phenolic extracts were combined and 150 ml. portions were shaken with 300 ml. diethyl ether. The aqueous layer that separated at this stage was collected and the phenolic phase was further extracted with ten 10 ml. portions of distilled water. The combined aqueous extracts so obtained were buffered with sodium-β-glycerophosphate at the rate of 0·5 g/100 ml., and the pH was brought to 7·4 by the addition of a drop or two of concentrated hydrochloric acid. The extract was then concentrated under reduced pressure (at 45° C) to about 70 ml. Traces of phenol were removed from the solution by shaking it with three 20 ml. portions of diethyl ether. Ether was then removed from the aqueous solution by distillation under reduced pressure and the solution was made to the desired volume (40–60 ml.). This extract was then suitably diluted for assay.

Plankton, which was preserved in 60% acetone, was extracted by steaming off the acetone, adding a few drops of a 1% solution of sodium cyanide, reducing the pH to 5·0 with 0·1 N-HCl and maintaining at 80° C for 20 min. The extracts were made to a desired volume, filtered through a Whatman no. 42 filter-paper and then diluted appropriately for assay.

Extracts were assayed with *Ochromonas malhamensis* (Ford, 1953) and with *Lactobacillus leichmannii*, ATCC 4797, by the method of Skeggs, Nepple, Valentik, Huff & Wright (1950) modified as described by Ford (1953).

It has been known for some time that, besides vitamin B_{12} (cyanocobalamin) itself, there exist in natural materials several analogues of vitamin B_{12} (see, for example, Kon, 1955). These are active to different extents for various organisms (Coates, Ford, Harrison, Kon & Porter, 1953; Droop, 1955) and organisms requiring 'vitamin B_{12}' differ in the extent to which they can utilize different analogues. Thus higher animals are generally narrowly specific in their utilization of vitamin B_{12} analogues but certain micro-organisms (e.g. *Escherichia coli*) are non-specific. Table I (compiled by Dr J. E. Ford) indicates the relative potencies of vitamin B_{12} analogues of natural occurrence for *Lactobacillus leichmannii* and *Ochromonas malhamensis*. Clearly, if the vitamin B_{12} activity of a given natural material is consistently higher for *Lactobacillus leichmannii* than for *Ochromonas malhamensis* and provided the higher *leichmannii* response is shown, by appropriate controls, not to be due to the presence of deoxyribosides in the material, it is reasonable to infer that vitamin B_{12} analogues active for *Lactobacillus leichmannii* but inactive for *Ochromonas malhamensis* are present in the material. Thus the presence of vitamin B_{12} analogues in natural materials can be detected by differential assays of this sort. Recovery tests (Table II) were performed by dividing a 4 l. sea-water sample into two 2 l. portions. One of these was extracted directly;

TABLE I. RELATIVE ACTIVITY OF VITAMIN B_{12} ANALOGUES AND DEOXYRIBOSIDES FOR ASSAY ORGANISMS

Factor	*Ochromonas malhamensis*	*Lactobacillus leichmannii*
Vitamin B_{12}	100	100
Vitamin $B_{12\,\mathrm{III}}$	40	50
Factor B	Inactive	Inactive
Factor A	Inactive	20
Pseudo-vitamin B_{12}	Inactive	50
Factor C	Inactive	Inactive
Deoxyribosides	Inactive	Active

TABLE II. RECOVERY FROM SEA WATER, BY 'PHENOL PASSAGE' TECHNIQUE, OF VITAMIN B_{12} ADDED TO IT

Sample	Vitamin B_{12} present. Portion extracted directly and assayed (μg/l.)	Vitamin B_{12} added to second portion (μg./l.)	Vitamin B_{12} found in second portion (μg/l.)	Percentage recovery
3½% saline	—	0·02	0·017	85
Unfiltered unpreserved sea water from 58° 40′ N., 6° 10′ W. (23 April)	0·003	0·004	0·006	75
Filtered preserved sea water from 60° N., 4° E. (1 June)	0·0003	0·004	0·0036	80
Unfiltered unpreserved sea water from 59° N., 1° E. (3 August)	0·0015	0·0006	0·002	83
Unfiltered unpreserved sea water from 61° N., 3° E. (10 August)	0·0035	0·001	0·0044	90
Unfiltered unpreserved sea water from 58° 40′ N., 6° 10′ W. (30 August)	0·0066	0·003	0·0094	90
Unfiltered unpreserved sea water from 59° N., 1° W. (3 October)	0·0033	0·004	0·0071	95

vitamin B_{12} in about the concentration expected in the sample was added to the other which was then extracted. The two extracts were then assayed and the percentage of recovered vitamin B_{12} determined.

RESULTS

The results obtained are shown in Table III. Values for filtered preserved samples are taken as indicating the concentration of vitamin B_{12} in the sea water; those for unfiltered unpreserved samples as an indication of the concentration which vitamin B_{12} might reach as a result of bacterial multiplication and action on detritus and organic matter contained in the water; those for the unfiltered preserved samples indicate the total amount of vitamin B_{12} present in the water (i.e. that present in solution and in suspended matter), they form controls to the corresponding unfiltered, unpreserved samples.

The vitamin B_{12} content of filtered preserved samples throughout the period under investigation varied between 0·006 μg/l. in coastal water (Wee Bankie, 56° 11′ N., 2° 08′ W., in January) and 0·0001 μg/l. in North Atlantic water (57° 55′ N., 17° W., in July); since the Seitz-filtered, bacteria-free, samples so far examined contained vitamin B_{12} in the same concentration as paper-filtered preserved samples collected at the same time, it is not unreasonable to assume that all the 'filtered preserved' values represent vitamin B_{12} present in solution in the sea. Droop (1954) gives a value of 0·005–0·01 μg vitamin B_{12}/l. for West Scottish coastal waters in February and March 1954, and Lewin (1954) found 0·01 μg vitamin B_{12}/l. in sea water of the Northwest Arm (Halifax, Nova Scotia) during the winter of 1952–53; their values agree adequately with that of 0·006 μg/l. for Wee Bankie in January and 0·016 μg/l. for Loch Fyne in March. Droop goes on to state that a cobalamin concentration of this order would not be a limiting factor in the growth of diatoms and other vitamin B_{12}-requiring protista. The cobalamin concentration of 0·0001 μg/l. recorded in North Atlantic water in July might, however, prove limiting, though vitamin B_{12} is probably only one of several factors (e.g. nitrate and phosphate concentrations) likely to be limiting at this time of the year.

Droop (1955, private communication) has also drawn attention to the fact that certain chrysomonads, and the only two diatoms known to require vitamin B_{12} (*Skeletonema costatum* and *Amphora perpusilla*) respond to analogues of vitamin B_{12} as well as to cyanocobalamin itself, and states 'it is likely that the analogues of vitamin B_{12} will have an importance equal to that of the vitamin in the ecology of the sea'. Differential assays of our sea-water extracts have shown the presence of vitamin B_{12} analogues in sea water. Water collected at Aberdeen Bay in January contained 0·006 μg vitamin B_{12}/l. when assayed with *Lactobacillus leichmannii*, and 0·004 μg/l. when assayed with *Ochromonas malhamensis*. Since vitamin B_{12} analogues are less

TABLE III. VITAMIN B_{12} CONTENT OF SEA WATER AND PLANKTON

NORTHERN NORTH SEA

	Locality and date							
Sample	59° N., 0° 40′ E. 29 May	60° 43′ N., 0° 30′ E. 3 June	59° N., 1° E. 3 Aug.	60° N., 00° 00′ 9 Aug.	58° 45′ N., 00° 00′ 7 Sept.	57° 15′ N., 1° 30′ W. 12 Sept.	59° 00′ N., 1° 00′ W. 3 Oct.	57° 00′ N., 1° 00′ W. 10 Oct.
Sea water								
Filtered preserved (μg/l.)	0·0002	0·0002	0·00013	0·0002	0·0007	0·0006	0·0009	0·0012
Unfiltered preserved (μg/l.)	0·00035	0·0002	0·00015	0·00018	0·001	0·005	—	—
Unfiltered unpreserved (μg/l.)	0·0005	0·0006	0·0015	0·004	0·0075	0·008	0·0083	0·0042
Plankton: Hensen net haul								
Total (μg)	0·6	1·2	0·2	0·07	—	—	—	—
Wet wt. (μg/g)	0·06	0·07	0·03	0·01	—	—	—	—
Plankton: Standard net haul								
Total (μg)	0·07	0·4	0·01	0·02	—	—	—	—
Wet wt. (μg/g)	0·03	0·06	0·01	0·003	—	—	—	—

BUTT OF LEWIS — NORWEGIAN DEEPS

	BUTT OF LEWIS				NORWEGIAN DEEPS		
	Locality and date				Locality and date		
Sample	58° 40′ N., 6° 10′ W. Feb.	58° 46′ N., 6° 30′ W. 4 Apr.	58° 40′ N., 6° 10′ W. 23 Apr.	58° 40′ N., 6° 10′ W. 30 Aug.	61° 01′ N., 3° 30′ E. 4 Apr.	60° 00′ N., 4° 00′ E. 1 June	61° 01′ N., 3° 30′ E. 10 Aug.
Sea water							
Filtered preserved (μg/l.)	0·002	0·001	0·0004	0·0006 0·00055 (Seitz-filtered)	0·002	0·0003	0·0005
Unfiltered preserved (μg/l.)	—	—	0·0008	0·0006	0·003	0·0002	0·0005
Unfiltered unpreserved (μg/l.)	0·004	0·006	0·003	0·0066	0·006	0·0005	0·004
Plankton: Hensen net haul							
Total (μg)	0·02	0·06	0·1	0·27	—	0·1	0·13
Wet wt. (μg/g)	0·05	0·05	0·07	0·04	—	0·03	0·08
Plankton: Standard net haul							
Total (μg)	0·005	0·02	0·5	0·05	—	0·006	0·03
Wet wt. (μg/g)	0·02	0·02	0·06	0·06	—	0·06	0·003
Seitz filter: used in filtering 4 l. sea water (μg)	—	—	—	0·0004	—	—	—

WEE BANKIE, LOCH FYNE, FAROE CHANNEL AND NORTH ATLANTIC

	Locality and date					
Sample	56° 11′ N., 2° 08′ W. Wee Bankie Jan.	Loch Fyne Mar.	60° 23′ N., 7° 49′ W. (F.C.) 23 Apr.	59° 56′ N., 7° 27′ W (F.C.) 31 Aug.	59° 54′ N., 14° 15′ W. (N. Atl.) 8 July	57° 55′ N., 17° 00′ W. (N. Atl.) 19 July
Sea water						
Filtered preserved (μg/l.)	0·006	0·16	0·0003	0·00073 0·0007 (Seitz-filtered)	0·00025	0·0001
Unfiltered preserved (μg/l.)	—	—	0·0002	0·0009	0·0003	0·0002
Unfiltered unpreserved (μg/l.)	—	—	0·008	0·005	0·005	0·003
Plankton: Hensen net haul						
Total (μg)	0·02	—	—	0·3	0·2	0·02
Wet wt. (μg/g)	—	—	—	0·04	0·03	0·005
Plankton: Standard net haul						
Total (μg)	0·01	—	—	0·08	0·01	0·002
Wet wt. (μg/g)	—	—	—	0·02	0·008	0·003
Filter-paper: used in filtering 4 l. sea water (μg)	—	—	—	0·0007	—	—
Seitz filter: used in filtering 4 l. sea water (μg)	—	—	—	0·0008	—	—

Notes. The vitamin B_{12}, found in the filter-paper (0·0007 μg) after filtering 4 l. of sea water through it, presumably represents the vitamin B_{12} present in suspended matter in the water. There would then be 0·0007/4, i.e. 0·00017, μg vitamin B_{12} present in suspended matter in each litre of sea water. If this value is added to that obtained for the filtered preserved water (0·00073 μg/l.) the sum tallies well with the value obtained for the unfiltered preserved sample, from which suspended matter had not been removed. A similar agreement was obtained with the Seitz-filtered and unfiltered preserved samples.

active for *Lactobacillus leichmannii* than vitamin B_{12} itself (e.g. factor A has only 20% of the activity of vitamin B_{12}) they are clearly present in this instance in a concentration greater than 0·002 μg/l. In the oceanic waters I have so far examined the concentration of analogues present when compared with that of vitamin B_{12} was very low, and a situation has not yet been encountered where the relative concentrations of vitamin B_{12} analogues and vitamin B_{12} were such as to limit the growth of organisms able to utilize vitamin B_{12} only, while permitting growth of organisms responding to vitamin B_{12} analogues and to vitamin B_{12}. In Table III only one value for vitamin B_{12} concentration is quoted for each sample from each station, since values obtained from *L. leichmannii* and *Ochromonas malhamensis* assays were not significantly different. When sea-water extracts used for routine assays were further concentrated and chromatographed cyanocobalamin alone could be detected. However, chromatograms of an extract of a 90 l. bulk of sea water showed that, besides vitamin B_{12}, factor B was also present.

TABLE IV. VITAMIN B_{12} ANALOGUES DETECTED IN MARINE ANIMALS

	Factor B	Factor A	Pseudo-vitamin B_{12}	Vitamin B_{12}
Haddock, gut contents	+	+	+	+
Cod, gut contents	+	0	+	+
Crangon allmani gut and contents	+	0	+	+

Ericson & Lewis (1953) have demonstrated that marine bacteria, isolated from sea water and from marine algae, are able to produce vitamin B_{12} factors. Similarly, the presence of such factors has now been shown chromatographically in the contents of the alimentary tract of various marine animals (Table IV), where they doubtless arise as a result of bacterial metabolism. Excretion by marine animals must contribute to some extent to the vitamin B_{12} analogues to be found in the sea.

The most obvious feature of the results to date is a seasonal variation in the vitamin B_{12} content of the waters examined; it is amply demonstrated in Fig. 1 for the three areas sampled. Although lack of time has made it impossible to obtain results for all three areas over a 12-month period, the similarity in seasonal trends in these areas is already apparent. Furthermore, the concentration of vitamin B_{12} in the few samples of North Atlantic and Faroe Channel water that have been examined was similar to the concentration in water from the other three areas at that time. From a winter maximum of about 0·002 μg vitamin B_{12}/l. the concentration fell to a level of about 0·0002 μg/l. by July and then gradually increased again to the winter level. Though there were slight temporal differences between the sequences in the three areas, the vitamin B_{12} content of the waters was broadly similar at any given time and none of these waters was characterized by a consistently higher vitamin B_{12} content than others. Thus in the biologically distinct areas so far examined a significant difference in the concentration of vitamin B_{12} has not been established.

The vitamin B_{12} content of the water decreased in March–April, the period during which plankton organisms were multiplying most rapidly. Figs. 2 and 3 show for the Butt of Lewis and Northern North Sea areas the total vitamin B_{12} content of Standard net and Hensen net plankton hauls taken at the same time as water samples. Though the volume of plankton taken must depend to some extent on weather conditions at the time, it can be seen that the quantity of vitamin B_{12} present in plankton was greatest when the vitamin B_{12} content of the water was lowest. Whether or not this was the result of a direct uptake of vitamin B_{12} from the sea water by diatoms is uncertain, since very little is known about the numbers of phytoplankton species that require vitamin B_{12} and the species, if any, that synthesize vitamin B_{12}, but the finding at least suggests the possibility. No estimate is as yet available of the relative contributions of the various factors affecting the regeneration of vitamin B_{12} in the sea during the late summer and autumn, though supposedly excretion by marine animals, bacterial action on detritus, and possibly mixing of surface waters with lower layers are all important in this respect.

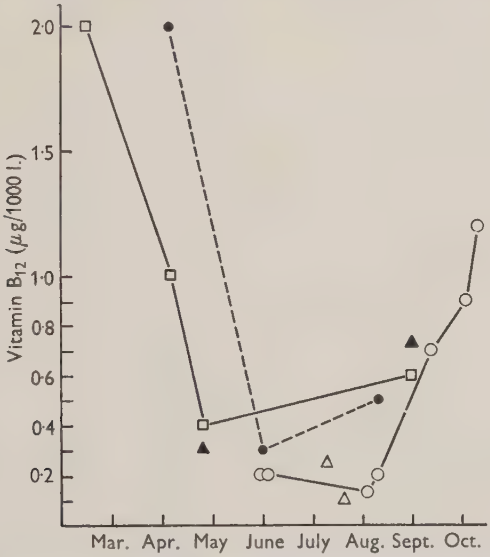

Fig. 1. Vitamin B_{12} content of filtered, preserved sea-water samples collected during 1955. □, Butt of Lewis; ●, Norwegian Deeps; ○, Northern North Sea; ▲, Faroe Channel; △, North Atlantic.

In untreated sea water (unfiltered unpreserved samples) the number of bacterial species present falls during the first 3–6 h of storage, and there then follows an increase of many hundred per cent of the surviving species

(ZoBell & Anderson, 1936). It can be seen from Table III that, although the unfiltered unpreserved samples contained more vitamin B_{12} than the unfiltered preserved controls, the difference was never very great. Observations were not made of types and numbers of bacteria that multiplied in the unfiltered unpreserved samples and it is unlikely that similar types multiplied in each sample, but at least some of those that multiplied had a definite, if comparatively restricted, ability to produce vitamin B_{12} under conditions which simulated the natural ones. The vitamin B_{12} content of the unfiltered unpreserved samples showed similar trends to those of the filtered preserved samples (Fig. 4), i.e. generally more vitamin B_{12} was produced in samples collected during autumn and winter than in samples collected during the summer.

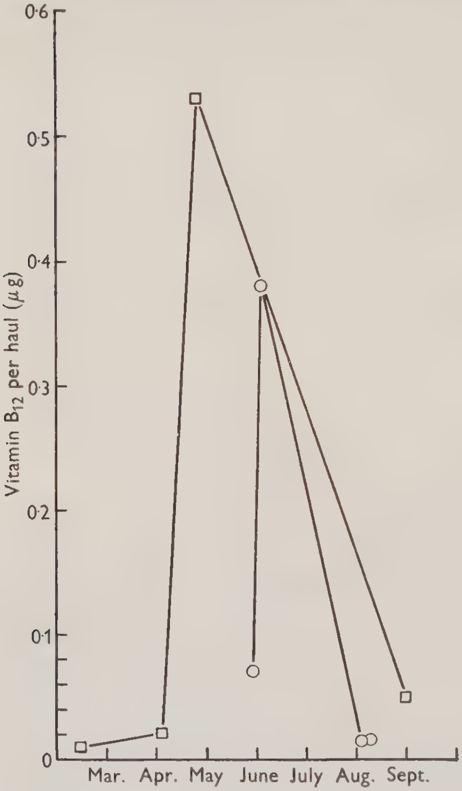

Fig. 2

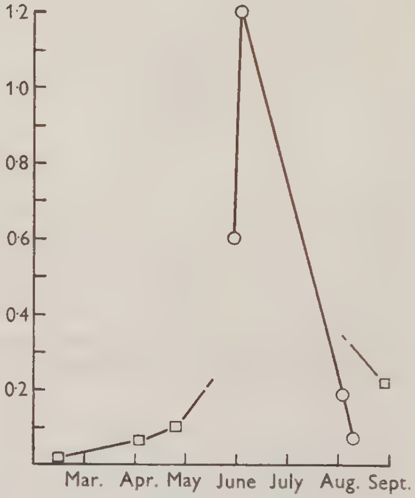

Fig. 3

Fig. 2. Vitamin B_{12} content of plankton taken in Standard net hauls during 1955. □, Butt of Lewis; ○, Northern North Sea.

Fig. 3. Vitamin B_{12} content of plankton taken in Hensen net hauls during 1955. □, Butt of Lewis; ○, Northern North Sea.

The physical conditions (such as relationships between solid surface area and volume) likely to control bacterial multiplication in untreated sea water stored in bottles were maintained constant from sample to sample. If one can assume that the vitamin B_{12} content of these samples was in some measure a reflexion of bacterial activity, it seems that under these conditions sea water collected during autumn and winter was more conducive to production of vitamin B_{12} by bacteria than water collected in summer.

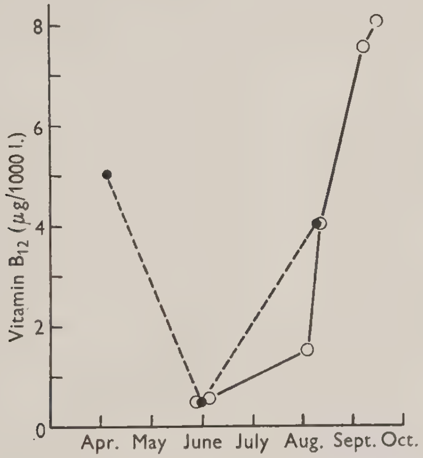

Fig. 4. Vitamin B_{12} content of unfiltered, unpreserved, sea-water samples collected during 1955. ○, Northern North Sea; ●, Norwegian Deeps.

The large zooplankton collections contained mainly *Calanus finmarchicus*, and their vitamin B_{12} content was 0·06–0·07 μg/g wet weight. Collections of *C. finmarchicus* alone contained 0·09 μg vitamin B_{12}/g wet weight (Table V). Another mixture of zooplankton, including the siphonophore *Lensia*, and the copepods *Rhincalanus*, *Pleuromamma*, and *Calanus finmarchicus*, gave 0·05 μg vitamin B_{12}/g wet weight. They could thus constitute an adequate source of vitamin B_{12} for higher marine forms. By comparison, the richest animal sources of vitamin B_{12} (e.g. cow liver) contain up to 1·3 μg vitamin B_{12}/g fresh tissue (Ford, Holdsworth & Porter, 1953).

There is not yet sufficient information to show whether species of the zooplankton from different sea-water masses differ in their vitamin B_{12} content. Indications are that they do not: thus *Meganyctiphanes norvegica* and *Euchaeta norvegica* both contained 0·05 μg vitamin B_{12}/g wet weight no matter where they were collected.

Phytoplankton contained much less vitamin B_{12} per unit wet weight than zooplankton. Thus samples of live phytoplankton in hauls were found to contain 0·02–0·03 μg vitamin B_{12}/g wet weight. The comparative richness of zooplankton may indicate a rapid rate of grazing; on the other hand, zooplankton might derive its vitamin B_{12} largely from a bacterial flora in the gut.

TABLE V. VITAMIN B_{12} IN MARINE INVERTEBRATES

			Activity expressed in terms of vitamin B_{12}. Wet weight (μg/g)	
Species	Organ	Analogues of vitamin B_{12} detected	*Escherichia coli*	*Ochromonas malhamensis*
Crangon allmani Kinahan	Whole animal	Factor B, pseudovitamin B_{12}	0·14	0·12
	Viscera	Factor B, pseudovitamin B_{12}	0·5	0·4
	Bodies	None	0·09	0·08
Pandalus bonnieri Caullery	Whole animal	Pseudovitamin B_{12}	0·14	0·11
	Gut and hepatopancreas	Pseudovitamin B_{12}	0·52	0·45
	Muscle	None	0·07	0·06
	Exoskeleton	None	0·13	0·09
Nephrops norvegicus L.	Exoskeleton	None	0·03	0·02
	Muscle	None	0·03	0·03
	Gills	None	0·03	0·02
	Alimentary canal and contents	Factor B and pseudovitamin B_{12}	0·08	0·05
	Digestive gland	Factor B and pseudovitamin B_{12}	0·12	0·11
Meganyctiphanes norvegica (M. Sars)	Whole animal	None	0·06	0·05
Euchaeta norvegica Boeck	Whole animal	None	0·05	0·06
Thysanoessa raschii (M. Sars)	Whole animal	None	0·08	0·07
Calanus finmarchicus (Gunnerus)	Whole animal	None	0·09	0·09
Aurelia spp. and *Ctenophores*	Whole animal	None	0·02	0·02

REMARKS

This work is exploratory and hence, of necessity, is only capable of giving pointers in new directions. No decisive conclusions are possible. Because of the time involved in the extraction procedure, results had to be based on single 4 l. samples from each station, i.e. there is no statistical basis for the values quoted. This drawback can best be overcome, and work of this nature generally facilitated, by acquiring marine micro-organisms that could be used for rapid direct assay of micronutrients in sea water. Furthermore, values obtained by direct assay of sea water are perhaps intrinsically more reliable than those obtained otherwise.

Besides the need for information on the concentration in sea water of vitamins, amino acids, and growth factors generally, the nutrition and synthetic powers of marine bacteria require examination, more especially of any forms associated with diatoms *in vivo* or inhabiting the alimentary tracts of marine animals. Only then can we construct a reasonable picture of the interplay between different factors, upon which productivity in the sea is ultimately dependent.

I am grateful to the Director of the Scottish Home Department Laboratory at Aberdeen for providing facilities for the collection of sea water and plankton samples, and to Drs R. Johnston and J. H. Fraser of that Laboratory for constructive discussion in the initiation and indeed throughout the course of this work. I am especially indebted to Dr Johnston for supervising the collection of samples.

I have derived much benefit from the interest, advice and critical approach during discussions of Drs L. R. Fisher, J. E. Ford, E. S. Holdsworth and S. K. Kon.

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SUMMARY

The vitamin B_{12} concentration in biologically distinct sea waters has been measured over a period of several months during 1955, a solvent-extraction technique being used to concentrate the vitamin and reduce the salt concentration in the water.

Similar seasonal changes in vitamin B_{12} content were found in water from the Butt of Lewis, Northern North Sea, and Norwegian Deeps, with a high winter level (about 0·002 μg/l.) and low summer level (about 0·0002 μg/l.). There was no evidence of a spatial variation in vitamin B_{12} concentration of the waters examined.

The vitamin B_{12} contents of plankton from hauls taken throughout the year (1955) at stations where water samples were collected, and of collections of individual zooplankton species were also determined and are reported on.

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THE BACTERIAL OXIDATION OF AMMONIA IN THE SEA

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(Text-figs. 1–3)

The final regeneration stages of the nitrogen cycle in the sea are believed to consist of the bacterial oxidation of ammonium-nitrogen to nitrite and finally nitrate-nitrogen. There have been many attempts to demonstrate the presence in the sea of marine counterparts to the terrestrial *Nitrosomonas* and *Nitrobacter*. (See Zobell, 1946, for bibliography of the earlier work.) Bacteria which can oxidize either ammonia or nitrite are easily demonstrated in samples of bottom material or water contaminated by land drainage or bottom deposits. On the other hand, there has been general failure to demonstrate the presence of such species in the upper layers of the open ocean.

Nevertheless, oceanographical evidence suggests that considerable nitrification occurs in these upper layers. There is no evidence that, under natural conditions, purely chemical catalysis occurs to any appreciable extent. The photo-oxidation of ammonia in sea water by ultra-violet light has been demonstrated (Zobell, 1933), but it is unlikely that this can be important except to a depth of about 1 m. The failure to demonstrate the presence of ammonia-oxidizing bacteria in the middle layers of open ocean water therefore leaves a serious gap in our knowledge of the nitrogen cycle in the sea. Zobell (1946) has suggested that some marine nitrifiers may exist which are of a very different physiological character from the strictly autotrophic terrestrial species, and that failure to demonstrate their presence might perhaps therefore be due to the use of unsuitable culture methods.

The present paper reports the results obtained during an investigation of the nitrifying behaviour of the surface water from the International Hydrographical Station E1. These studies were commenced whilst working at the Plymouth Laboratory and were continued and extended at the Marine Biology Station of the University College of North Wales, Menai Bridge.

METHODS

All cultures were grown in 250 ml. Pyrex conical flasks capped with small beakers. The medium used was essentially that employed by Carey (1938). For initial enrichment cultures 10 g of acid-washed fine sand and 1 g calcium carbonate were dispensed in each flask and sterilized by autoclaving at 10 lb. for 20 min; 40 ml. samples of sea water were added followed by aseptic additions of phosphate ($0\cdot1 \times 10^{-3}$ M) and ammonium chloride ($2\cdot0 \times 10^{-3}$ M) from sterile stock solutions of the required strength. For making sterile media for subsequent cultures, 40 ml. of aged sea water were added to each flask, together with the sand and calcium carbonate, and the whole sterilized by autoclaving at 5 lb. for 30 min. All cultures were incubated at 22° C in the dark.

Experience showed that little nitrification occurred without a solid phase in the medium. Further tests demonstrated that sand alone was not very effective as a solid phase. Carbonate was much more effective, and in the presence of calcium carbonate an addition of sand had little further effect. In the later experiments the sand was therefore omitted.

The progress of ammonia oxidation was followed by withdrawing samples at intervals and estimating for nitrite using the method of Rider & Mellon (1946). The cultures were agitated before samples were withdrawn to suspend the carbonate evenly. After development of the colour, the intensity was read by means of a Harvey absorptiometer using Ilford Filters no. 604. Nitrite was determined by means of a calibration curve prepared by assaying solutions of sodium nitrite of known strengths containing suspended calcium carbonate in the same proportions as in the cultures. The carbonate dissolves in the acid medium used for the diazotization, but this and the final coupling reaction are pH sensitive, and the calibration curve was therefore prepared using samples analogous to those obtained from cultures.

EXPERIMENTAL

In the preliminary experiments the nitrifying behaviour of surface water from the International Hydrographical Station E1 was investigated by setting up enrichment cultures. Surface water was collected after the development of the thermocline and dispensed in 40 ml. amounts in sterile flasks containing sand and calcium carbonate with additions of phosphate and ammonium chloride. Periodic sampling showed that no formation of nitrite could be detected in this series of cultures in up to 80 days' incubation, and if any oxidation of ammonia was occurring it must have been extremely slow. No tests were made for the formation of nitrate since most previous investigators agreed that nitrite formation became maximal before appreciable oxidation to nitrate occurred (von Brant, Rakestraw & Renn, 1937).

These preliminary results largely confirmed the much earlier findings of Harvey (1926) with surface-water samples from the same station. They further suggested that it was not solely the lack of a solid phase in the culture medium that was inhibiting ammonia oxidation as suggested by Cooper (1948).

In a further attempt to set up enrichment cultures a series of water samples were incubated as before except that an additional addendum of 1 ml. of a uni-algal (but not bacteria free) stock culture of a diatom was made to each flask. After an initial induction period of 35 days nitrite formation commenced in all cultures of this series and continued vigorously until a maximum value was reached after some 60 days. The observation that the presence of plankton material allowed nitrification to proceed was reminiscent of the results of Carey (1938).

Another series of enrichment cultures was set up to identify the origin of the ammonia-oxidizing bacteria. These cultures showed that no nitrite formation occurred if the sea-water samples were sterilized by autoclaving regardless of whether the diatom addendum was sterile or not. Further, with freshly collected unsterilized water samples, the progress of nitrite formation showed little difference whether the diatom suspension together with associated bacteria was added in a fully viable condition or after having been sterilized by autoclaving. It therefore seemed certain that ammonia-oxidizing bacteria were present in the water samples and not in the crude diatom culture. Further confirmation of this was obtained by the use of a bacteria-free culture of a marine diatom. The addition of this also allowed nitrite formation to occur in non-sterile samples of the sea water, whereas nitrite formation could not be detected after 80 days' incubation in any control culture set up with sterilized sea water or without additions of the diatom suspension.

Attempts were next made to subculture the nitrifying organism. 1 ml. samples of the initial enrichment cultures described above were transferred to sterile medium without the addition of the diatom suspension. The formation of nitrite proceeded in all the subcultures without the marked induction period observed in the original enrichment cultures but at a much reduced rate. In subsequent subcultures the rate of nitrite formation was further successively reduced. Progress curves for the production of nitrite in initial enrichment cultures and in a subsequent subculture are shown in Fig. 1. The concentration of nitrite in the enrichment cultures varied exponentially with time. This is typical of an autocatalytic reaction and is the effect that might be expected with a proliferating catalytic organism. On the other hand, the concentration of nitrite in the subsequent subcultures was directly proportional to the time of incubation.

An investigation was made of the effect of variations in the size of inocula on the kinetics of nitrite production in subcultures without the addition of diatoms. Within limits, the rate of nitrite production varied directly with the size of inocula and remained linear. With the largest inocula, however, the production of nitrite, though initially linear, tended to give way to an exponential rate. The kinetics of nitrite formation in subcultures without added diatoms and the results of variations in the inocula size in such subcultures suggested the presence of a non-proliferating but enzymically active bacterial population.

The addition of a sterilized diatom suspension to a subculture inhibited the production of nitrite for periods of up to 30 days. Thereafter rapid exponential formation of nitrite occurred. A hot-water extract of diatoms from which the insoluble debris had been removed by centrifuging was considerably less inhibitory, the induction period in this case lasting only 2 or 3 days. Such extracts seemed equally effective as whole cells in ultimately promoting exponential nitrite formation in subcultures. In control experiments only negligible production of nitrite occurred in cultures containing sterile diatom suspensions or hot-water diatom extracts without the addition of ammonium-nitrogen. It therefore seemed probable that the ammonia-oxidizing bacteria in the water samples needed for growth some factor or factors supplied by the diatoms.

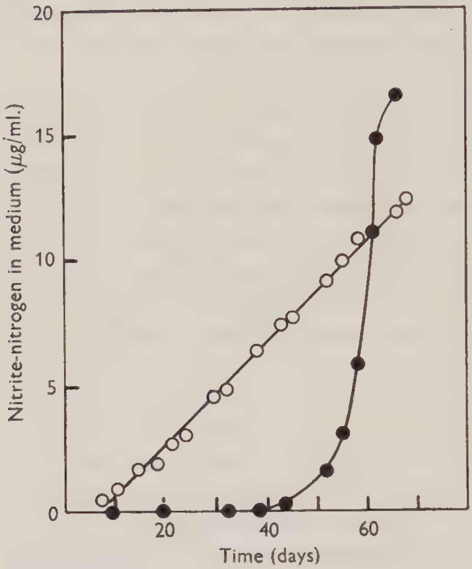

Fig. 1. Progress curve for nitrite production in medium made up with E 1 water. –●–●–, Initial enrichment culture with addition of diatoms; –○–○–, subculture without addition of diatoms.

An examination was made of the effects of the initial concentration of ammonia on the rate of nitrite production in non-proliferating cultures. Variable results were obtained, and it proved impossible to obtain repeatable data for a concentration-rate curve. However, the results indicated that initial concentrations of over 0·01 M ammonium-nitrogen were increasingly inhibitory to the rate of nitrite formation.

Quastel, Scholefield & Stevenson (1952) have reported the direct production of nitrite from organic nitrogen without the intermediate formation of ammonia. The results of control cultures and the recoveries of nitrogen as nitrite, which agreed well with the original addition of ammonia, made it unlikely that the nitrite could in any case be formed entirely from the organic nitrogen of the diatoms. It remained possible that appreciable nitrite might be formed directly from organic nitrogen synthesized by the heterotrophic bacterial flora present. All attempts to demonstrate the presence of any organism capable of producing nitrite from pyruvic acid oxime gave negative results.

The experiments were continued at the Marine Biology Station, Menai Bridge. An investigation was first made of the nitrifying behaviour of freshly collected water from the Menai Straits. These water samples showed vigorous formation of nitrite when incubated with calcium carbonate and suitably enriched with phosphate and ammonium-nitrogen. Furthermore, when subcultures were made from these enrichment cultures to sterile media made up from Menai Straits water without the addition of diatoms, the exponential production of nitrite persisted without diminishing throughout numerous successive subcultures. This behaviour was in marked contrast to that shown by cultures set up in E 1 water. The sea water used in these and all subsequent subcultures was 'aged' water which had been filtered through Whatman no. 2 filter-paper immediately after collection and then allowed to stand for at least 1 month in the dark.

These results agreed with those reported by many previous workers, for there seems to be general agreement that nitrifying bacteria occur in coastal waters contaminated by land drainage and bottom deposits. In conjunction with the results reported above, which demonstrated the presence of ammonia-oxidizing bacteria in E 1 water which apparently required for growth a factor or factors in diatoms, there were two possible interpretations of the observed experimental observations. Either the ammonia-oxidizing organisms which were present in the two waters were of two types with differing nutritional requirements or, alternatively, the Menai Straits water contained the factors supplied to E 1 water by a diatom suspension. Experiments were therefore made to investigate these possibilities.

Cultures were set up in sterile media of identical composition made up with either aged Menai Straits water or a sample of aged E 1 water (membrane-filtered immediately after collection). These cultures, together with the relevant controls, were inoculated from an enrichment culture set up with Menai Straits water. The results of such an experiment are shown in Fig. 2. It will be seen that the cultures in medium made up with Menai Straits water exhibited an exponential production of nitrite and therefore presumably grew well. In contrast, the cultures in medium made up with E 1 water exhibited a slow linear production of nitrite. This behaviour was similar to the subcultures from the original E 1 enrichment cultures. This similarity was emphasized by the subsequent observation that the addition of a sterile diatom suspension allowed an exponential production of nitrite in cultures made up with E 1 water and inoculated from Menai Straits enrichment cultures. It therefore seemed likely that there was a difference between the two water masses with respect to their ability to support the growth of the ammonia-oxidizing bacteria, there being no evidence to suggest that the ammonia-oxidizing bacteria from the two localities had different nutritional requirements.

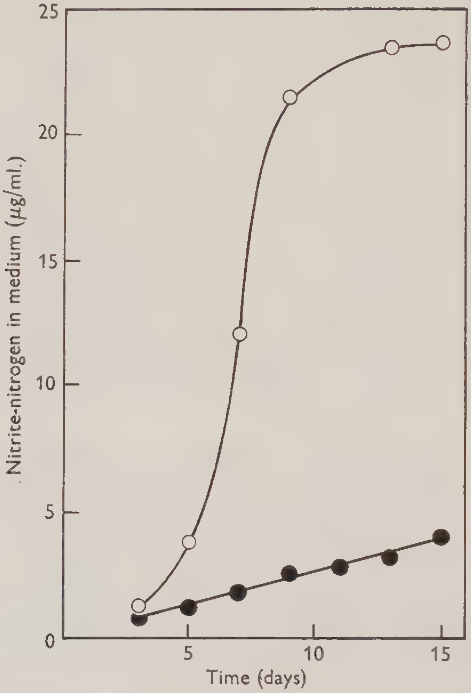

Fig. 2. Progress curve for nitrite production after subcultures from Menai Straits enrichment culture. –○—○–, Medium made up with Menai Straits water; –●—●–, medium made up with E 1 water.

An examination of the two waters showed markedly different contents of iron (estimated by the method of Cooper, 1935). The sample of E 1 water used contained a barely detectable amount of iron, whereas water from the Menai Straits had an iron content of the order of 20–30 μg/l. Meiklejohn has shown that the terrestrial species of *Nitrosomonas* and *Nitrobacter* require considerable amounts of iron for growth. In addition, it seemed certain that some iron was being added with the diatoms since these had been grown in iron-rich medium and are known to accumulate considerable quantities of iron. It therefore appeared possible that variations in the iron content might account for the variations in the ability of the two waters to support the growth of the marine nitrifiers. Experiments showed (Fig. 3) that the addition of iron to subcultures in medium made up with E 1 water allowed an exponential production of nitrite. The concentrations of nitrite in Fig. 3 are plotted on a logarithmic scale and the slope of the graphs is presumably a measure of the growth rate of the nitrifying bacteria. It will be seen that the additions of iron to E 1 water allowed growth at a rate comparable to that in Menai Straits water.

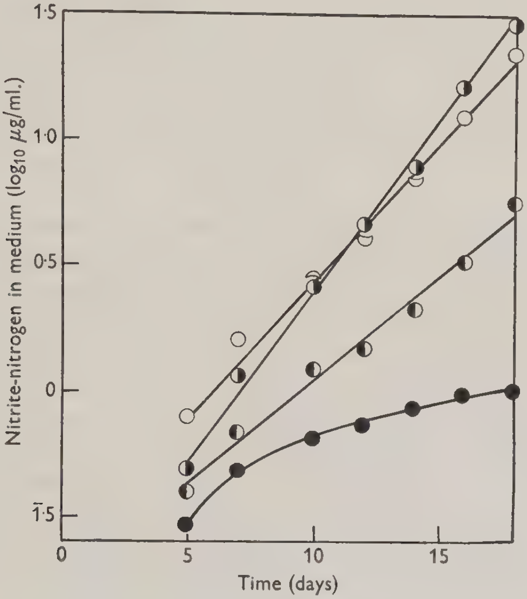

Fig. 3. Progress curves for nitrite production after subcultures from a Menai Straits enrichment culture. -●—●-, E 1 water medium, no additions; -○—○-, Menai Straits water medium, no additions; -◐—◐-, E 1 water medium with addition of diatoms; -◑—◑-, E 1 water medium with addition of 100 µg./l. iron.

Medium made up with E 1 water with the addition of iron supported growth of the ammonia-oxidizing bacteria in numerous successive subcultures and after ten subcultures there was no indication of any diminished growth rate. Subsequent successive subcultures without the addition of iron showed decreasing rates of growth and after three or four subcultures of this type the production of nitrite again became proportional to time and characteristic of a non-proliferating bacterial population. It is noticeable that several subcultures are necessary for the effect to become apparent in this case, in contrast to the linear production of nitrite in the first subcultures from Menai Straits water to E 1 water without the addition of a diatom suspension. This is probably due to the much lower content of iron in the latter parent cultures.

The iron content of Menai Straits water can be greatly reduced by filtration through a membrane filter. The growth of the ammonia-oxidizing bacteria in medium made up with this filtered water was similar to that observed in medium made up with E 1 water.

It has not been found possible to determine the optimum concentration of iron for the growth of the marine nitrifiers since analysis showed relatively considerable quantities of iron in the calcium carbonate, it being impossible to assess how much of this would be available to the bacteria. No success was obtained in an attempt to find an iron-free solid substratum that would allow optimum growth. It is nevertheless possible to deduce that relatively high concentrations of iron are required for optimum growth since the addition of 100 μg/l. to E 1 water caused a higher rate of nitrite production than occurred in a Menai Straits water control which contained at least 30 μg/l. (Fig. 3).

DISCUSSION

The results reported above make it probable that the amount of iron present in a body of sea water may be the rate-limiting factor controlling the bacterial oxidation of ammonia. The amount of iron which can exist in solution in sea water is exceedingly small and it would be expected that open ocean water containing relatively little suspended matter would be low in available iron content. On the other hand, coastal waters and those contaminated by land drainage or bottom material invariably contain considerable amounts of suspended matter and might be expected to be relatively rich in available iron. It therefore seems very possible that the numerous reports of failure to detect ammonia-oxidizing bacteria in open ocean water may often be due to failure to provide an adequate supply of iron and not to the absence of the catalysing bacteria. It is noteworthy that Carey (1938) found that certain waters naturally rich in phytoplankton (or after the addition of phytoplankton townetting) allowed the vigorous oxidation of ammonia, whereas in similar samples containing a preponderance of zooplankton little nitrification occurred. Judging by the result obtained by Cooper (1935) on the iron content of phytoplankton and zooplankton, Carey's observation could be due to the relatively higher iron content of the phytoplankton.

If this interpretation is correct, considerable bacterial oxidation of ammonia may occur in the upper layers of the open ocean as suggested by the oceanographical evidence. The actual rate of oxidation in nature would be expected to be much lower than that observed in culture, due not only to relatively low levels of iron but also to low substrate concentrations. It is obvious, however, that such waters might fail to show appreciable nitrifying activity if cultured without the addition of iron. A wide survey of the nitrifying activity of freshly collected open ocean waters using the culture techniques described in this paper is required to test this possibility.

Details of the identity and nature of the catalysing organism must await its isolation. Several previous workers have reported the failure of attempted isolation studies. Waksman, Hotchkiss & Carey (1933) noted the formation of nitrite on silica gel plates inoculated from their enrichment cultures, but apparently no observable growth occurred. This is reminiscent of the production of nitrite without proliferation of the catalysing organism noted during the present studies. It seems probable therefore that any silica gel medium used in isolation studies with the marine nitrifiers will have to be well enriched with iron. There is, however, no evidence to suggest that the marine nitrifiers are of different physiological type to their terrestrial counterparts. The results obtained by von Brandt *et al.* (1937), by Carey (1938), and in the present studies show that the onset of the oxidation of ammonia is delayed in cultures containing organic matter. *Nitrosomonas* is a strict autotroph and its growth is inhibited by organic matter. During the induction period presumably the heterotrophic flora present is removing the inhibitory organic matter. Furthermore, the observation of von Brant *et al.* that oxidation of nitrite does not commence until all the ammonia has been oxidized is typical of the behaviour of *Nitrobacter*, the growth of which is inhibited by quite low concentrations of ammonia. The inhibition of ammonia oxidation, with increasing ammonia concentrations of the order noted in the present studies, is typical of *Nitrosomonas*.

It is interesting that the optimum iron concentration in culture lies well above the solubility of the ferric ion. Similar results were obtained by Meiklejohn (1953). Working with the terrestrial *Nitrosomonas* she reported optimum iron concentrations of the order of 6 mg/l. At the high pH values employed for culture these iron concentrations are certainly in excess of the solubility of iron. These observations and the present studies suggest, therefore, that these organisms are capable of, and may be dependent on, utilizing particulate iron. The nitrifying bacteria show a very marked tendency to attach themselves to particles of the solid phase in the culture medium. Lees & Quastel (1946) have shown that ammonia is not oxidized in soil cultures unless it is absorbed on to the surface of the soil colloids. The adherence tendencies of these bacteria may thus be necessitated by their method of mobilization of ammonia. If, however, considerable quantities of iron are required, the bulk of which is in particulate form, it is difficult to see how this could be mobilized unless physical contact between the cells and the particles was achieved. The attachment propensities of these organisms may thus be due in part to the necessity of their mobilizing iron in particulate form.

Part of the work reported above was performed at the Plymouth Laboratory. It is a great pleasure to thank the Director and Staff for making freely available the facilities of the laboratory, especially to Dr H. W. Harvey, F.R.S., for his continual interest and encouragement, and Mr F. A. J. Armstrong for collecting water samples.

SUMMARY

The failure of a sample of E 1 water to oxidize added ammonium-nitrogen to nitrite-nitrogen has been shown to be due, not to the absence of catalytic bacteria in the water, but rather to the lack of suitable nutrient conditions for proliferation of the nitrifying bacteria.

The addition of sterile diatoms or sterile hot water extracts of diatom cells to E 1 water allowed the proliferation of the nitrifying organisms with concurrent oxidation of ammonium-nitrogen to nitrite-nitrogen.

Water from the Menai Straits allows the proliferation of its naturally occurring nitrifying flora without additions of diatom material. These organisms, however, will not grow in E 1 water unless diatom material is added.

Additions of iron allow the nitrifying bacteria from Menai Straits water to grow in E 1 water. These additions of iron have a quantitative effect comparable to additions of diatom material.

It is suggested that the many previous failures to demonstrate the presence of nitrifying bacteria in the middle layers of open ocean waters may be due not to their absence but to failure to supply sufficient iron for appreciable growth to occur in culture. This may bring the bacteriological evidence more in line with the oceanographical evidence which suggests that considerable formation of nitrite occurs in these layers.

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EVIDENCE OF SELF-FERTILIZATION IN CERTAIN SPECIES OF BARNACLES

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Isolated individuals of certain species of cirripedes are known to remain unfertilized at the time when the majority of contiguous individuals are carrying egg masses. From a very large number of observations on both *Balanus balanoides* (L.) and *Elminius modestus* Darwin (Crisp, 1950, 1956) there remains little doubt that in these two species copulation is necessary before eggs are brought into the mantle cavity where they are fertilized. Though fewer field observations have been made, *Balanus crenatus* Bruguière appears to behave similarly, isolated specimens grown on raft-exposed panels never bearing fertilized egg masses (Crisp, 1950; Barnes, unpublished observation). *B. balanus* (L.) (= *B. porcatus* da Costa) is also in all probability an obligatory cross-fertilizing hermaphrodite, though the available evidence does not exclude the possibility of self-fertilization in rare instances (Crisp, 1954; Barnes & Barnes, 1954).

It appeared possible to one of us that obligatory cross-fertilization might be the rule for all cirripedes, and some ecological consequences of this were pointed out by Crisp (1950). However, we have independently come to the conclusion that certain species, though they may normally cross-fertilize whenever this is possible, can also bear apparently viable egg masses without copulation having taken place.

EVIDENCE FOR SELF-FERTILIZATION IN *CHTHAMALUS STELLATUS*

Under natural conditions it is exceedingly difficult to find an individual of *Elminius modestus*, *Balanus balanoides* or *B. crenatus* fertilized when separated by a distance of 5 cm or more from its neighbour. This critical distance represents the maximum extension of the penis. Since the possibility usually exists that another individual might be carried into the vicinity of an experimentally isolated barnacle on the shell of a wandering mollusc, any rare exception may be attributed to such an accident. In some circumstances such a remote possibility may be entirely excluded, as for example in the case of *Elminius modestus* which has been shown to be cross-fertilizing by carrying out experiments on individuals introduced into an area that was not at the time populated by this species (Crisp, 1956). Nevertheless, even when the possibility of individuals approaching accidentally is not entirely excluded, the artificially isolated individuals are almost invariably found to be without fertilized egg masses in these three species. It is therefore reasonable to conclude that if in such an experiment with another species a significant proportion of isolated individuals become gravid, the majority of them, if not all, must have deposited their eggs in the mantle cavity without having copulated with another individual.

TABLE I. SELF-FERTILIZATION IN *CHTHAMALUS STELLATUS*

Date	Locality	Distance between individuals (cm)	No. of individuals in sample	Percentage fertilized	Percentage fertilized among controls
24. vi. 54	Millport	2·5– 5·0	20	45	96
		5·0– 7·5	20	40	
		7·5–10·0	20	40	
		>10·0	20	45	
12. vii. 54	Millport	2·5– 5·0	20	55	48
		5·0– 7·5	20	60	
		7·5–10·0	20	50	
		>10·0	20	32	
4. viii. 54	Millport	2·5– 5·0	20	55	55
		5·0– 7·5	20	50	
		7·5–10·0	20	60	
		>10·0	20	30	
8. vi. 52	Aberffraw	1–3	57	53	57
		3–5	20	50	52
		5	13	23	28
20. vii. 52	Aberffraw	1–3	10	80	70
		3–5	14	86	71
		>5	37	41	50
22. vi. 52	Black Rock* nr. Portmadoc	>7	14	36	43
7. vi. 53	New Brighton*	>10	50	18	74
29. vi. 53	New Brighton*	2–5	10	20	100
		>10	8	0	90
5. vii. 53	Aberffraw	>10	7	57	55

* Naturally occurring individuals which were not experimentally isolated.

This conclusion applies with special force in the case of *Chthamalus* growing sparsely near high-water mark in areas where it is not very abundant, for *Chthamalus* is only found on mollusc shells when it is extremely common on the rocks throughout the intertidal zone.

Table I gives the percentage of individuals carrying egg masses, compared with the percentage found among a large number of controls gathered from the same piece of rock at the same level. Control individuals were in all cases in physical contact with one or more other specimens of mature size. Great care is necessary in choosing control individuals to ensure that they are drawn from the same type of environment since tide level, aspect, and access to surf can profoundly influence the fecundity. At Millport, the results refer to naturally isolated individuals of mature age. In the North Wales experiments the individuals were in most cases isolated by removing all others within a given distance prior to April, since breeding does not start until May, although in some cases naturally isolated individuals were taken in the sample.

Table 1 clearly shows that while at some places isolated individuals appear to be just as fertile as contiguous ones, in other instances, as for example at New Brighton, there is evidence that isolated individuals have not been fertilized to the same extent as contiguous individuals.

There is some evidence to show that oviposition may be delayed in isolated as compared with contiguous specimens. The observations at Aberffraw on 9 June 1952 were made early in the breeding season at a time when the first brood was developing in the mantle cavity. Although there was only a small percentage fertilized in both isolated and contiguous individuals, the latter were at a slightly later stage of development. Thus the percentage of the population which had reached a given stage of development was always greater in the contiguous than the isolated populations. Similarly, on 29 June 1953 at New Brighton, where a very great disparity existed between isolated and contiguous individuals, the latter had just produced a second brood and the embryos were all in an early stage of development. At Millport, where conditions are less favourable for this warm water species, the percentage fertilized in June was much higher in the contiguous individuals. The evidence as a whole is consistent with the view that oviposition may take place without insemination by another individual if the ovaries remain unfertilized for some time after reaching a ripe condition. It follows, therefore, that unless these eggs develop parthenogenetically, self-fertilization must take place.

In addition to these observations, fertilized isolated individuals have also been met with from time to time. These are more obvious in areas where the species is not very abundant, as in the north of Scotland. Examples of apparently self-fertilized individuals have been seen at W. Loch Tarbert, Argyll 1 August 1953 at about M.H.W.S.T.L., at 'the spur' west of Dunnet Head, Caithness, and by Dr A. J. Southward (personal communication) at Stenness, Esha Ness, Shetland. Individuals grown in isolation on raft-exposed panels at Millport have also been found to contain ripe egg masses.

VIABILITY OF EGGS WHICH ARE NOT CROSS-FERTILIZED

Eggs produced without copulation appear to be less viable than normally fertilized eggs. Occasionally in egg masses of many species, groups of ova may be found which do not develop, but remain in the primitive unsegmented condition. Such eggs are probably unfertilized and are voided with the hatched nauplii when the latter are fully developed. It was noticed that a considerable proportion, sometimes as many as half, of the egg masses from isolated individuals contained unsegmented ova together with developing ova.

In other cases abortive development took place, giving rise to segmented eggs with an abnormal appearance. For example, certain of the eggs examined from isolated individuals collected at Aberffraw on 8 June 1952 were difficult to classify as a stage in normal development, and one pair of egg masses appeared to be decomposing. It is therefore possible that the earlier state of development of broods from isolated individuals may not indicate later oviposition, but be due to abnormally slow development. Further evidence supporting this hypothesis is that at Millport during the late summer, whilst all stages of embryos were found in contiguous individuals indicating the development of successive broods in the population, only late stages were found in the isolated individuals. Slow development in the latter would prevent a further generation from being developed since eggs are not brought into the mantle cavity, even when ripe, in the presence of an earlier brood Crisp & Davies, 1955).

Nevertheless, viable nauplii can be produced by isolated individuals, and indeed in every observation at Aberffraw recorded in Table I, and in the observation at Black Rock, at least one isolated individual contained nauplii which were fully developed and able to hatch.

At Millport undeveloped and degenerate egg masses in the mantle cavities of isolated individuals appear to be common earlier in the breeding season. In the early summer most of the eggs in the mantle cavities of isolated individuals were incompletely developed and in many cases the whole egg mass had begun to degenerate *in situ*; on being touched with a needle the egg mass broke down into an oily suspension of tissue. This is perhaps related to the fact that in such regions where the species is approaching its limit of distribution, only in late summer are the environmental conditions suitable for the species to reproduce. Thus even the early broods developed in contiguous individuals, although giving apparently normal nauplii which on teasing out will swim actively, do not give rise to any shore settlement. However, the broods produced from isolated individuals in the late summer appear completely normal; the nauplii hatch readily and swim vigorously. Similar temperature conditions apply in the Isle of Man, where the settlement of *Chthamalus* is confined to a period in late summer or autumn (Southward & Crisp, 1956).

The efficiency of self-fertilization also appears to be less than that of cross-fertilization as regards the number of normal embryos produced. Counts of normal embryos in isolated and contiguous individuals of several size groups show a greater mean number (*t*-test) in the latter.

OVIPOSITION IN THE DRY STATE

While in the dry state *Chthamalus stellatus* remains closed and does not protrude either the cirri or the penis. Copulation is therefore impossible under these conditions. However, there appears to be no reason why oviposition should not occur, provided that copulation were not a necessary stimulus, just as moulting can take place with the shell closed (Darwin, 1854). A number of stones covered with *Chthamalus* were stored in a moist warm place and sampled from time to time to determine the stage of development of the embryos. The egg masses developed normally, the earlier stages disappearing progressively from the population with the passage of time. Development was completed in 15–16 days at 18–20° C and in 21–24 days at 14–15° C. The initial divisions of ova to form a blastoderm covering the yolk cell (stage 4, see Crisp, 1954) normally occupy less than 15% of the total time of development. Occasionally such stages were found long after this period had elapsed. In one individual egg masses at the eight cell stage (stage 3) were found in addition to fully developed egg masses 9 days after the eggs had been maintained at 18° C out of water. In another two, egg masses with blastoderm covering an undivided or partially divided yolk cell (stage 4–5) were found 14 days after the eggs had been maintained out of water at a temperature of 15° C. In all three instances the eggs appeared to be healthy. These occurrences were exceptional and did not invalidate conclusions on rate of development being drawn from the experiment. Unless the rate of development can be greatly reduced by some factor other than temperature (e.g. water loss or oxygen lack) the conclusion must be drawn that oviposition in the dry state may occur under such conditions.

Evidence for Self-fertilization in other Species

Single specimens of *Verruca stroemia* on shells of *Modiolus* are often dredged from Beaumaris bay. These apparently isolated specimens have been found on several occasions to contain developing egg masses. The shells of *Modiolus* are very sparsely scattered over the sea bed in this area (Baird, private communication) and the possibility of two individuals on separate shells lying close together must be very small. At Millport, *Verruca stroemia* gives one brood each year; fertilization takes place in late December and early January; the nauplii are shed in late February and early March, a little before those of *Balanus balanoides* and *B. crenatus*. Isolated and contiguous individuals on stones collected at extreme low water were examined for egg masses. By mid-January all individuals contained developing egg masses. There was little trace of any degeneration in those from isolated individuals, but eggs showing delayed development were often found with normally developing embryos in the same animal. This again suggests that cross-fertilization is more efficient. By mid-February some actively swimming nauplii could be obtained from most individuals. There was no apparent difference between nauplii derived from isolated and from contiguous barnacles. Further, barnacles which had been isolated by 5 cm and maintained on a raft for many months contained ripe egg masses with normal embryos.

Balanus perforatus is a large intertidal species with a thick calcareous base which remains if an individual dies or is knocked off. Consequently, if an individual appears isolated it is easy to check whether another, no longer present, had formerly occupied a site sufficiently close to it for cross-fertilization. The species is rarely found on the shells of littoral molluscs. In parts of north Devon individuals isolated by a distance of 10 cm or more may be found in the same locality as groups of three or four together.

Table II gives the results of an investigation on a reef at Saunton Sands, North Devon. There is no doubt that in mid-August, when the observations were made, individuals situated more than 10 cm from each other not only can carry egg masses, but also are as fertile as contiguous specimens. However, the embryos of individuals grouped together were generally in a more advanced state of development.

TABLE II. SELF-FERTILIZATION IN *BALANUS PERFORATUS*

	Percentage with egg masses	Percentage with eggs at Stage 9 or below	Percentage with eggs at Stage 10, 11 and 12	Percentage with fully developed eggs
Contiguous individuals	66	14	15	37
Individuals separated by more than 10 cm	67	33	12	21

MECHANISM OF DEVELOPMENT OF EGGS OF ISOLATED INDIVIDUALS

It is not possible to decide except by a cytological investigation whether the eggs in the mantle cavity of isolated individuals are fertilized or have been developed parthenogenetically. The abnormal development of the eggs in some instances suggests the possibility of parthenogenesis. On the other hand, the testes and ovaries are ripe simultaneously and the penis is suitably placed for self-fertilization by discharging spermatozoa into the mantle cavity. Both *Chthamalus stellatus* and *Verruca stroemia*, and probably *Balanus perforatus*, are normally cross-fertilizing; *Chthamalus* has frequently been observed to copulate both in the laboratory and in the sea. However, in those species in which the development of eggs in isolated individuals occurs at all, it appears to take place with such regularity that it can hardly be considered as an unnatural or exceptional mode of reproduction. Moreover, the process is probably of some value to at least two species, for isolated individuals of *Chthamalus* are by no means uncommon towards high tide mark, while in deep water isolated *Verruca* are often encountered. If the eggs of such individuals were developing parthenogenetically, however, a further process would be required subsequently to bring about duplication of the chromosomes. The simpler hypothesis is, therefore, that self-fertilization takes place.

The possibility that water-borne spermatozoa can effect fertilization at a considerable distance from their origin must also be considered. The spermatozoa of barnacles, unlike those of most other crustacea, are flagellated. Direct microscopic observations, as well as electron micrographs, show that there is very considerable variation morphologically within the group. Spermatozoa of *Balanus balanoides*, and probably of other species of the genus, are spindle-shaped and very elongated; under the electron microscope there appears to be no difference between the extremities (Barnes & Dainty, unpublished results). Spermatozoa of *Chthamalus* and *Verruca*, on the other hand, have a definite head and tail which are distinguishable even under the light microscope. The spermatozoa of barnacles are usually immobile in the seminal fluid, but have been observed to become extremely active, both in *Balanus balanoides* and *Elminius modestus*, either after being discharged through the penis into the mantle cavity of another individual or as a result of treatment with chelating agents such as ethylene diamine tetra-acetic acid (Crisp, unpublished observation; cf. Fujii, Utida & Mizuno, 1955). In the last two species the spermatozoa are not otherwise readily activated, though an occasional movement may sometimes be seen when they are mixed with sea water. In *Verruca* and *Chthamalus*, however, the spermatozoa more frequently show activity when squeezed from the seminal vesicles into sea water. This greater spontaneous activity of the spermatozoa in sea water of those species in which fertilization in isolated individuals has been shown to be common might suggest that water-borne spermatozoa are responsible. However, if this were so it would be expected that the incidence of fertilization in isolated individuals separated by more than 3–5 cm, the distance reached by the extended penis, would be a function of the distance of isolation. This is not the case; the incidence is sometimes equal to that of individuals lying within 3 cm of each other, and sometimes lower. There is never any indication of a gradual reduction of the incidence of fertilization with increasing distance of isolation, such as would be expected if water-borne spermatozoa were responsible. This difference in the activity and morphology of the spermatozoa may, nevertheless, be related to the capacity for self-fertilization.

Further, oviposition normally takes place in barnacles as a result of the stimulus of copulation and does not occur when embryos are developing in the mantle cavity (Crisp & Davies, 1955). Eggs awaiting fertilization are therefore rarely, if ever, found in the mantle cavity of cross-fertilizing species, nor have they been observed during the examination of many egg masses of *Chthamalus*, *Verruca* and *Balanus perforatus*. Similarly, seminal fluid in most species is emitted only as a result of stimulation during copulation; it is only rarely discharged directly into the water. When it comes in contact with sea water the seminal fluid usually coagulates and is dispersed only with difficulty. Water-borne insemination therefore appears most improbable since it requires that two processes (unstimulated oviposition and unstimulated seminal discharge), which on present evidence very seldom occur in nature, should occur simultaneously. The mechanism can be considered feasible only on the assumption that the barnacle acting as a female collects spermatozoa in sufficient quantity to provide an oviposition stimulus. The apparent oviposition and development of eggs in the mantle cavity of individuals which have not been immersed in water for long periods shows that water-borne spermatozoa are not essential.

If in these species self-fertilization accounts for the fecundity of isolated specimens, it is pertinent to inquire what is the stimulus leading to oviposition when it is not provided by copulation. In *Chthamalus* the ovaries give rise to a succession of broods, and should oviposition be held back because the barnacle is not suitably stimulated by insemination the ovaries may become very full of ripe eggs. It is possible that under these conditions the threshold of the oviposition stimulus becomes so reduced that movements of the barnacle's own penis in the mantle cavity are sufficient to cause eggs to be released, and seminal fluid is then simultaneously discharged.

If the continued ripeness of the ovary were the stimulus leading to self-fertilization, one would expect the process to be more in evidence where the species was growing rapidly and had abundant food supplies. Individuals kept out of water for long periods, or those growing in unfavourable environments, would not be so likely to self-fertilize as isolated specimens on rocky coasts. The environment at New Brighton is sheltered, silty and unfavourable to the species, whereas it thrives on the exposed west shores of Anglesey and Caernarvonshire (Crisp & Knight-Jones, 1954). The greater readiness with which self-fertilization occurs in the latter area at Aberffraw and Black Rock compared with New Brighton might therefore be explained in terms of nutrition. Similarly, in the earlier part of the summer more planktonic food would probably be available and the lower temperatures would cause basal metabolism to make less call on food reserves; hence self-fertilization might occur more readily at this season than later on, when reserves are becoming spent.

Summary

1. Isolated individuals of *Chthamalus stellatus*, *Verruca stroemia* and *Balanus perforatus* often carry apparently fertilized egg masses. Fertilization by water-borne spermatozoa seems improbable because spermatozoa are not normally shed into the water by barnacles, nor are unfertilized eggs usually present in the mantle cavity except during copulation. Either self-fertilization or parthenogenesis must therefore be assumed to have taken place.

2. Eggs found in isolated individuals are often less viable than normal eggs. Nevertheless, they are sometimes capable of full development, and hatch to give swimming nauplius larvae.

3. The percentage of normal embryos is significantly less in egg masses of isolated individuals, than in egg masses of contiguous individuals.

4. Oviposition can probably occur in *Chthamalus stellatus* while it is out of water.

5. There is some evidence that self-fertilization takes place more readily in *C. stellatus* when external conditions are most suitable for breeding. Self-fertilization might therefore be ascribed to a lowering of the threshold of the oviposition stimulus resulting from the continued presence of mature eggs in the ovary.

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ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

THE OXIDATION OF L-AMINO ACIDS BY *MYTILUS EDULIS*

By **H. Blaschko** and **D. B. Hope**

Biochem. J., Vol. 62, 1956, pp. 335–9

The enzymic oxidation of amino acids by preparations of the digestive gland of *Mytilus edulis* was described some time ago. A study has now been carried out of the stereospecificity of the oxidase; it has been established that the enzyme is a L-amino acid oxidase. The enzyme acts upon a great number of L-amino acids. Methionine was the substrate most rapidly oxidized. The enzyme was found to be associated with insoluble tissue constituents; in sucrose homogenates the enzymic activity could be sedimented by centrifugation.

A similar but less active preparation was obtained from the digestive gland of *Cyprina islandica*. Earlier findings of a D-amino acid oxidase in the digestive gland of *Helix aspersa* were confirmed. H.B.

EGG-CAPSULE PROTEINS OF SELACHIANS AND TROUT

By **C. H. Brown**

Quart. J. micr. Sci., Vol. 96, 1955, pp. 483–8

The material of the egg-capsules of selachians and the chorion of trout eggs has been examined by physical, chemical and histochemical methods. The material of the egg-capsules has been found to be a quinone-tanned protein. The chorion of trout eggs is not quinone tanned but its formation and chemical behaviour allies it with the invertebrate cuticular proteins rather than with the vertebrate keratins. C.H.B.

ON THE HORMONAL CONTROL OF WATER BALANCE IN *CARCINUS*

By **D. B. Carlisle**

Pubbl. Staz. zool. Napoli, Vol. 27, 1955, pp. 227–31

Experiments on *Carcinus* indicate that the uptake of water at moulting and the water and osmotic balance throughout the rest of the moult cycle are under the control of a hormone secreted by the X organ-sinus gland complex. This factor appears to be distinct from the hormones controlling moulting and from the gonadotrophic hormone. D.B.C.

MUSCLE MORPHOGENESIS IN PRIMITIVE GASTROPODS AND ITS RELATION TO TORSION

By **Doris R. Crofts**

Proc. zool. Soc. Lond., Vol. 125, 1955, pp. 711–50

The retractor muscle of the pre-torsional larvae of the four primitive gastropods investigated is a single group of cells attached to the right side of the shell apex in *Haliotis*, *Patella* and *Patina*, but half-way along the right side in *Calliostoma*. The anterior attachments on the right side are retractors of the velum and mantle: those on the left belong to sickle-shaped cells curving over the gut and are retractors of the left side of the velum and foot rudiment.

The primary ontogenetic cause of torsion appears to be the asymmetry of this *single* pre-torsional muscle, which may have been an ancestral mutation, instead of asymmetry in *two* ancestral larval muscles, a left one related to the foot and a right one related to the head, as in Garstang's hypothesis (1929).

The first phase of torsion is rapid, but varies in time and extent in the genera. The main cause of the variation may be the position of shell attachment of the retractor, with other contributory factors. The second torsion phase is slow and is mainly due to differential growth. The pedal musculature of the right side begins development during this phase, and at the completion of 180° rotation its shell attachment on the definitive right side is roughly the same size as that of the original larval retractor, now on the left. The definitive right shell muscle, which, contrary to earlier supposition, becomes the columellar muscle of *Calliostoma* and the hypertrophied one of *Haliotis*, may be a pair to the pre-torsional retractor, delayed in development. The two muscles may represent the two ancestral retractor muscles of gastropods, whose scars were discovered by Knight (1947) in *Bellerophon* and *Sinuites*.

D.R.C.

ENVIRONMENTAL FACTORS GOVERNING THE INFECTION OF MUSSELS, *MYTILUS EDULIS*, BY *MYTILICOLA INTESTINALIS*

By **B. T. Hepper**

Fish. Invest., Lond., Ser. 2, Vol. 20, No. 3, 1956

The importance of *Mytilicola*, its spread to and around the shores of Britain, its method of dispersal, life history and hosts are briefly discussed.

A number of extensive field surveys produced evidence to show that mussels raised from the sea bed, or in fast moving water or at either end of an estuary are less heavily infected than bottom-dwelling mussels, those in slow moving water or those in the middle region of an estuary respectively. These conclusions are supported by the results of field trials.

It is concluded that control of the parasite is possible by using culture methods similar to those practised in France and Italy on stakes, fences or ropes. By growing mussels on selected sites in fast moving water or at the brackish-water end of an estuary, good quality mussels may be cultured on the flat. In addition to restricting the level of infection, such culture methods appear to provide conditions which enable the mussels to withstand parasitization without serious loss of condition. B.T.H.

THE NERVOUS SYSTEM OF THE EPHYRA LARVA OF *AURELLIA AURITA*

By **G. A. Horridge**

Quart. J. micr. Sci., Vol. 97, 1956, pp. 59–74

Methylene-blue preparations of living ephyra larvae show two nerve nets, of which one, of bipolar cells, overlies only the radial and circular muscle sheets and is identified with the giant fibre net (Shäfer's nerve net) of the adult. The other net, which consists of smaller, multipolar cells, spreads over both surfaces of the animal: it is identified with the physiological conducting system which co-ordinates the movement of a single arm with the directed pointing of the mouth in the feeding reaction. It is called 'the diffuse nerve net'. The 'spasm', in which the animal folds up, stops beating, and sinks through the water, is considered to be an extension of the feeding reaction round the disc, and is shown to be co-ordinated by the diffuse net. The independence of the locomotory beat from the feeding responses and from the spasm is thus explained by the dual histological arrangement. A double innervation of the muscle is also suggested. Both nerve nets connect with the marginal ganglia, where four types of nerve cell have been described. A partial explanation of the action of the ganglion is possible, with an input of excitation from the diffuse net (which affects the rate of the rhythm) and an output to the giant fibre net which initiates the beat. This scheme gives a histological foundation for Romanes's old experiments, in which he demonstrated the difference between 'excitational' and 'contractional' continuity across the bell of the adult, and in which he found an indirect action on the rhythm following stimulation at points far from the marginal ganglia. G.A.H.

INACTIVATION ENZYMATIQUE D'UNE SUBSTANCE CHROMACTIVE DES INSECTES ET DES CRUSTACÉS

By **Francis Knowles, David Carlisle and Marie Dupont-Raabe**

C.R. Acad. Sci., Paris, Vol. 242, 1956, p. 825

Enzymic studies on the A substance of the eye-stalk of *Leander* suggest that this may be a polypeptide. D.B.C.

EXPERIMENTAL FEEDING OF THE COPEPOD *CALANUS FINMARCHICUS* (GUNNER) ON PHYTOPLANKTON CULTURES LABELLED WITH RADIO-ACTIVE CARBON (^{14}C).

By S. M. Marshall and A. P. Orr

Pap. Mar. Biol. Oceanogr., Deep-Sea Res., suppl. to Vol. 3, 1955, pp. 110–14

Cultures of the diatom *Skeletonema costatum* and the flagellates *Cryptomonas* sp. and *Syracosphaera carterae* were grown using ^{14}C as carbonate in the culture medium. The cultures were then used in feeding experiments on female *Calanus* in order to measure the uptake and percentage assimilated. The volume filtered varied from less than 1 to about 40 ml. in 24 h. and the amount of ^{14}C assimilated from 53–78 %. The results are in agreement with those from similar experiments done with ^{32}P. S.M.M., A.P.O.

COMPARISON OF THE LEVEL OF CHLORIDE REGULATION BY *NEREIS DIVERSICOLOR* IN DIFFERENT PARTS OF ITS GEOGRAPHICAL RANGE

By R. I. Smith

Biol. Bull., Woods Hole, Vol. 109, 1955, pp. 453–74

This paper summarizes data obtained on the level of coelomic-fluid chloride in *Nereis diversicolor* adapted to a series of dilutions of sea water and to fresh water. The populations studied have been collected in marine-dominated habitats (Millport and Kingsbridge Estuary), regions of stable low salinity (Gulf of Finland and the Isefjord, Denmark), and from typically estuarine conditions of variable and low salinity (River Tamar). Ecological findings have been reported in the *Journal of the Marine Biological Association* and elsewhere. Despite the wide differences in salinity and its pattern of variation in the several habitats studied, populations of *N. diversicolor* from different areas were found to show no significant differences in the level of coelomic chloride regulation after adaptation to low salinities. This level is thus not diagnostic of physiologically distinct races of *N. diversicolor*, although the ability to tolerate fresh water or sudden salinity changes may, upon further study, prove to differ in populations from regions of unlike salinity. R.I.S.

BOOK REVIEW

THE PROTOZOA, SARCODINA

By Margaret W. Jepps

Oliver and Boyd, London and Edinburgh. 183 pp. 1956

Dr Jepps has written this excellent book in a lucid style, and she has selected and prepared a fine series of illustrations. The book is a welcome addition to the meagre list of good monographs on the Protozoa. It is designed especially for senior students in zoology, and as such it provides a valuable supplement to laboratory work. In her introduction the author gives a competent summary of protozoan cellular behaviour, especially with reference to the cytoplasm. Although the reader is referred to appropriate listings in the index, a more detailed discussion of the nucleus would have enhanced the value of the introduction. After reading the book I was left with the impression that the author had neglected some of the more recent research on problems of protozoan physiology and biochemistry. One should be reminded, however, of the unavoidable lapse of time between submission of a manuscript to a publisher and its final publication. The emphasis on the flexible and tenuous nature of systems of protozoan classification is commendable. The inclusion of the slime moulds helps to establish the author's broad concept of unity within the Protista. The general format of the book is attractive, and the well-chosen bibliography and detailed index serve to increase the book's usefulness to all students of protozoology.

E. R. NOBLE

MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

Report of the Council for 1955–56

The Council have great pleasure in reporting that Prof. A. V. Hill, C.H., O.B.E., F.R.S., was elected President of the Association in June in place of Prof. Sir James Gray, Kt., C.B.E., M.C., F.R.S., who had served for the preceding ten years.

The Council wish to record their deep appreciation of the many services rendered to the Association by Sir James Gray during his long term of office as President, and are glad to report that he will continue to serve on the Council as Governor representing the Royal Society. Sir James Gray has been elected a Vice-President of the Association.

The Council have to record with regret the death of Mr D. J. Matthews, a former member of the scientific staff of the Plymouth laboratory, and of Dr A. G. Lowndes who was a regular visiting research worker at Plymouth for many years.

The Council and Officers

Four ordinary meetings of Council were held during the year, three in the rooms of the Royal Society and one at Plymouth. At these the average attendance was seventeen. The Association is indebted to the Council of the Royal Society for the use of its rooms.

During the year Mr N. B. Marshall has been appointed to succeed Dr H. W. Parker as Governor representing the Zoological Society of London.

The Plymouth Laboratory

The passage between the north building and the constant temperature and radioactive substances laboratories has been closed in with a glass roof and door. This has greatly improved the conditions for those working in these two small laboratories.

During the year the roof of the old library building has been relaid with new asphalt.

New Sea-Water Reservoirs

The excavation of the new sea-water reservoirs in the yard between the north and south buildings began in October 1955, and the work is progressing steadily. It has been decided to postpone the building of the new outside circulation tanks until the winter of 1956–57.

Dr D. P. Wilson has given much time to the planning of this scheme which, when completed, will enable all tanks used for research purposes to receive a supply of pure sea water from the new reservoirs, which will together hold about 30,000 gallons. The existing reservoirs, which have up till now supplied research tanks as well as the aquarium, will then only be used for the latter.

The Aquarium

The Aquarium has been well attended by the public throughout the year, but the takings in the summer were lower than usual owing to the exceptionally fine weather. In January and February the aquarium was closed for four weeks to enable four tanks, including the two largest, to be emptied for repair of the rockwork.

During the spring and early summer improvements were made to the circulation in most of the tanks. Wide-bore ebonite pipes were installed in corners and other inconspicuous places to carry much of the inflowing water down to the bottom, instead of discharging it only into the surface layers. This has noticeably improved the health of some of the fishes and is undoubtedly responsible for the absence of the usual warm weather troubles during a summer in which the water reached higher temperatures than it had for several years.

A further twenty picture labels have been painted by Miss Jenefer Peter so that few of the species shown are now without such an aid to identification by the visitor.

Research Ships

During the year the three research vessels have been in regular and continuous service.

During her annual survey it was found that the hull of the *Sarsia* was in excellent condition.

A new trawl winch has been installed in the *Sula*. This is a more powerful winch than that originally supplied with the ship and is proving most satisfactory. Certain modifications have also been made during the year to give increased accommodation in the engine room, in the wheel house and on deck.

A new Kelvin Ricardo 7½ h.p. engine has been fitted in the *Gammarus* to replace the engine on the port side, which had been in service since 1929.

Staff

Dr D. P. Wilson has been promoted to Senior Principal Scientific Officer on grounds of individual merit as from 1 July 1955.

Dr B. C. Abbott joined the staff of the Plymouth laboratory as Principal Scientific Officer on 1 September 1955.

Dr H. G. Vevers, M.B.E., left the staff of the Plymouth laboratory on

31 August 1955 to take up his appointment as Curator of the Aquarium and Director's Assistant on the staff of the Zoological Society of London.

Mr N. A. Holme has succeeded Dr Vevers as Bursar at the Plymouth laboratory.

Dr T. I. Shaw joined the staff of the Plymouth laboratory at the beginning of November 1955 on a special temporary appointment.

Dr J. A. C. Nicol accompanied cruises of R.R.S. *Discovery II* in October and November 1955, and February and March 1956 by kind invitation of the Director of the National Institute of Oceanography, to do research on luminescence in *Pyrosoma* and other oceanic animals.

Mr F. A. J. Armstrong has been elected an Associate of the Royal Institute of Chemistry.

Mr F. S. Russell, C.B.E., F.R.S., attended the International Conference on Marine Biological Laboratories held in Rome in April 1955, under the auspices of the International Union of Biological Sciences. He also visited the Stazione Zoologica at Naples.

Dr D. P. Wilson attended a symposium on marine biology held at the Scripps Institution of Oceanography at La Jolla in March 1956.

Mr N. A. Holme attended the meeting of the International Council for the Exploration of the Sea in Copenhagen in October 1955.

Miss D. Ballantine spent a month at the East African Fisheries Research Laboratory at Zanzibar by arrangement with the Colonial Office during February and March 1956, while on leave in Africa.

Occupation of Tables

The following one hundred and forty-three workers have occupied tables at the Plymouth Laboratory during the year:

E. ADAMS, Plymouth (Library).
Miss M. N. E. ADAMS, Southampton (Culture of phytoplankton).
R. D. ADAMS, Cambridge (Structure of the continental shelf).
J. ARMSTRONG, London (Nerve cells of molluscs).
D. C. ARNOLD, St Andrews (Holothuria).
Miss D. E. ASHHURST, Oxford (Blood groups in fish).
Dr DAPHNE ATKINS, Plymouth (Ciliary mechanisms of brachiopods, *Phoronis*, etc.).
A. L. BARETS, Paris (Muscle receptors of fishes).
G. T. BARLOW, California (Calibration of underwater photometer).
P. R. O. BARNETT, Derby (Library).
Miss G. BEESON, Oxford (General).
Dr L. G. E. BELL, London (Development of *Ciona*).
Dr JOYCE A. BENTLEY, Aberdeen (Algal culture).
Miss D. BEXON, Nottingham (Algae).
F. BEYER, Oslo (Hydrography and zooplankton).
E. J. BINYON, London (Library).
J. BLAGDEN, Cambridge (Interpretation of echo-sounding records).
Miss J. L. BLOOM, Exeter (General).

Dr B. P. BODEN, La Jolla (Submarine illumination).
A. D. BONEY, Plymouth (Culture of red Algae).
Prof. R. BUCHSBAUM, Pittsburg (Marine ecology and photography).
Dr P. C. CALDWELL, Beit Memorial Fellow (Physiology of crab muscle).
Mrs P. A. CALDWELL, Plymouth (*Spartina*).
Dr J. D. CARTHY, London (Library).
Miss Y. M. CHAMBERLAIN, London (Marine Algae).
Dr P. N. J. CHIPPERFIELD, Brixham (Library).
J. C. CLEVERLY, Cambridge (Structure of the continental shelf).
Dr H. A. COLE, Burnham-on-Crouch (Lynher and Tamar oyster fishery).
J. W. COLES, London (Nematodes parasitic on and associated with seaweeds).
R. J. CONOVER, Southampton and U.S.A. (Cruise on R.V. *Sarsia*).
Mr and Mrs E. COOPER-WILLIS, Wales (Drawings of marine organisms).
K. COPELAND, London (Electronic equipment).
Dr E. D. S. CORNER, International Paints Research Fellow (Effects of toxic substances on marine organisms).
C. A. COSWAY, Torquay (Library).
Dr T. J. LE COSQUINO DE BUSSY, The Hague (Waterblooms).
Miss W. A. M. COURTNEY, London (Respiration of cirratulids).
Dr D. J. CRISP, Bangor (Library).
D. CURRY, Pinner, Middlesex (Geology of English Channel).
Dr R. PHILLIPS DALES, London (Fat storage and metabolism in polychaetes).
E. W. DAWSON, Cambridge (Behaviour of lamellibranchs).
A. A. DAY, Cambridge (Structure of the continental shelf).
R. D. DESHPANDE, Reading (Physiology of Trochidae).
P. S. B. DIGBY, London (Copepods).
Miss E. J. DIMELOW, Reading (Functional morphology of *Antedon*).
D. A. DORSETT, London (Feeding of *Polydora ciliata*).
Mme M. DUPONT-RAABE, Paris (Colour change in crustaceans).
Dr MARIA FELINSKA, Oundle (Marine ciliates).
Dr L. R. FISHER, Reading (Vitamin A in plankton animals).
Cdr R. H. C. FRAMPTON, R.N. (Rtd.), Plymouth (Library).
R. F. H. FREEMAN, London (Behaviour of *Scrobicularia*).
Dr VERA FRETTER, Reading (Library).
Dr D. N. GANGULY, Calcutta (General).
J. B. GILPIN-BROWN, Bristol (Nervous system of *Nereis*).
Dr SYLVIA J. GILPIN-BROWN, Bristol (Behaviour of littoral gastropods).
A. GLANVILLE, London (Library).
Dr D. R. GLASSON, Plymouth (Library).
C. K. GODDARD, St Andrews (Endocrine glands of elasmobranchs).
R. U. GOODING, Cambridge (Parasitic crustaceans).
Prof. A. GRAHAM, Reading (Library).
P. GRAY, Plymouth (Library).
A. V. GRIMSTONE, Cambridge (Ciliates).
Dr E. R. GUILER, Tasmania (Intertidal ecology).
Y. HALIM, Alexandria (Chemistry of sea water).
Prof. A. C. HARDY, F.R.S., Oxford (Drawings of marine animals).
Prof. F. T. HAXO, La Jolla (Algal physiology and biochemistry).
B. T. HEPPER, Burnham-on-Crouch (Lynher and Tamar oyster fishery).
Prof. A. V. HILL, F.R.S., London (Heat production in crustacean nerve).
Dr M. N. HILL, Cambridge (Structure of the continental shelf).

G. E. Hobson, London (Annelid phosphagens).
Prof. A. L. Hodgkin, F.R.S., Cambridge (Nerve conduction in *Loligo*).
J. V. Howarth, London (Heat production in crustacean nerve).
Dr A. F. W. Hughes, Cambridge (Cytology of fish neurones).
O. D. Hunt, Newton Ferrers (Library).
D. C. Ingram, Oxford (General).
L. A. J. Jackman, Paignton (Library).
F. J. Jeffery, Plymouth (Library).
H. S. Jeffries, Bradford-on-Avon (Library).
Dr C. H. Jellard, Plymouth (Library).
Miss P. M. Jenkin, Bristol (Library).
Dr Elizabeth M. Kampa, La Jolla (Submarine illumination).
Dr G. Y. Kennedy, Sheffield (Porphyrins and chlorophyll pigments).
A. K. Kent, Aberystwyth (Paper electrophoresis and endocrinology).
Dr R. D. Keynes, Cambridge (Nervous conduction in *Loligo*).
Prof. W. B. R. King, F.R.S., Cambridge (Geology of the English Channel).
M. C. Kingwell, South Brent (Library).
Sir Frances G. W. Knowles, Bart., Marlborough (Colour change in crustaceans).
G. A. Knox, New Zealand (Shore ecology; library).
Dr P. L. Kramp, Copenhagen (Medusae).
C. V. Kurian, Travancore (Larvae of decapod crustaceans).
Dr D. Lacy, London (Green gland of crustaceans).
Dr Marie V. Lebour, Plymouth (Decapod crustaceans).
Dr J. Llewellyn, Birmingham (Trematode ectoparasites of fishes).
Prof. O. E. Lowenstein, F.R.S., Birmingham (Sensory physiology of elasmobranchs).
Dr A. G. Lowndes (the late), Plymouth (Mineral content of sea water).
Dr J. Lowy, Belfast (Mechanical properties of *Mytilus* muscle).
Miss A. C. Maddocks, Oxford (General).
Dr A. M. A. Maghraby, Alexandria (Plankton).
Dr S. B. Markowski, London (Marine Algae and zooplankton).
A. L. Martin, London (Feeding of amphipod crustaceans).
Mrs J. B. Matthews, St Merryn (Library).
Dr D. M. Maynard, Cambridge and U.S.A. (Histology of decapod nervous system).
G. F. Mees, Leiden (Feeding of *Zeus faber*).
P. L. Miller, Cambridge (Shore Diptera).
Miss F. M. Molloy, London (Digestive system of crustaceans).
Dr J. E. Morton, London (Biology of *Lasaea rubra*).
Dr R. W. Murray, Birmingham (Ampullae of Lorenzini).
Dr Margaret Naylor, London (Algal cytology).
Miss M. E. Needler, London and Toronto (Neuromuscular physiology of sea anemones).
D. Nichols, Oxford (Feeding of sea urchins).
Prof. E. R. Noble, Santa Barbara (Protozoan parasites of fishes).
Mrs E. Oldfield, Guildford (Embryology of *Lasaea rubra*).
Dr C. F. A. Pantin, F.R.S., Cambridge (Neuromuscular physiology of *Actinia*).
L. B. Pradhan, Madras (Mackerel fisheries).
G. L. Rao, Madras (Fishing gear and methods).
Dr W. J. Rees, London (E. T. Browne manuscripts).
Miss B. Rickard, Plymouth (Library).
Dr F. H. Rigler, Toronto (Ion exchange by plant cells).
Miss E. A. Robson, Cambridge (Neuromuscular physiology of *Actinia*).

Dr D. M. Ross, London (Neuromuscular physiology of sea anemones).
M. J. S. Rudwick, Cambridge (Brachiopods).
Dr W. M. S. Russell, London (Humane killing of crabs).
A. Sharples, Plymouth (Library).
G. Smith, Aberystwyth and Perth, Australia (Estuarine fucoids).
R. E. Soeriaatmadja, Indonesia (Chemistry of sea water).
M. K. Soong, Malaya (Library).
Dr A. J. Southward, D.S.I.R. (Distribution and breeding of marine animals).
Dr Eve C. Southward, Plymouth (Polychaetes).
B. W. Sparrow, Newton Ferrers (Library).
Miss F. A. Stanbury, Plymouth (*Cladophora*).
Dr F. C. Stott, Ewell (Food canal and haemal network in holothurians).
Prof. K. Strøm, Oslo (Geomorphology of Devon and Cornwall).
O. Suddaby, Plymouth (Library).
Dr Olive S. Tattersall, Hayling Island (Breeding cycles in mysids).
Miss A. M. Taylor, Manchester (Rock pool Algae; *Ascophyllum*).
M. Unni, Madras (Fishing gear).
A. J. Vinson, Nottingham (Algae).
G. E. Walster, Plymouth (Library).
Prof. G. P. Wells, F.R.S., London (*Arenicola*).
Dr Mary Whitear, London (Nervous system of tunicates).
Dr D. I. Williamson, Port Erin (Larvae of pagurid crustaceans).
Miss M. J. Wood, London (Culture of flagellates).
H. V. Wyatt, Plymouth (Biology of *Calyptraea*).
Prof. J. Z. Young, F.R.S., London (Nerve cells of molluscs).

Among the many scientists who have visited Plymouth during the year to see the general work of the laboratory and to discuss problems with members of the scientific staff, the following have come from overseas: Dr Reynolds, U.S.A.; Dr R. W. Hiatt, Hawaii; Dr P. Dohrn, Naples; H. C. Butcher, Penang; Dr J. C. Medcof, Canada; Dr Helen I. Battle, Ontario; Dr Tham Ah Kow, Singapore; Dr T. Imai, Japan; R. G. Collins, Nigeria; G. R. Fish, Malaya; Prof. C. Smith, Nova Scotia; Sidney W. Fox, Tallahassee; Dr D. Davenport, Santa Barbara; P. Bougis, Banyuls-sur-Mer; Prof. Ricardo Jorge, Lisbon; Dr R. Revelle, La Jolla; Dr R. C. Swan, New York; Dr G. A. C. Herklots, Trinidad; J. C. Yaldwyn, New Zealand; Dr X. M. Aubert, Belgium; Dr Antoine Dohrn, Naples; R. Beary, U.S.A.; Dr D. E. Hurley, New Zealand; Dr C. Mary John, Travancore; Dr A. C. Redfield, Woods Hole; Dr W. Schmitz, Germany.

The Danish Fishery Research Vessel *Dana* visited Plymouth on her return voyage from Greenland waters in August 1955. Among those on board were Dr P. Hansen, Mr F. Hermann, Mr J. Nielsen and Dr Mario Ruivo.

The Easter Vacation Courses were conducted by Mr G. M. Spooner and Mr P. G. Corbin, and were attended by forty students from the following Universities and University Colleges: Oxford, Cambridge, Glasgow, London, Durham, Liverpool, Sheffield, Southampton, Exeter, Aberystwyth, Galway, Chelsea Polytechnic and Regent Street Polytechnic.

Also during the Easter Vacation Mr K. W. Wilkes brought a party of five boys from Harrow School and Mr E. C. Turner brought seven boys from Buckhurst Hill County High School.

Dr G. E. Newell and Dr J. E. Morton conducted a course in September for sixteen students from Queen Mary College.

Scientific Work of the Plymouth Laboratory Staff

Sea Water and Plankton

Dr L. H. N. Cooper has continued his work on the inter-relation of Arctic climate, the deep circulation of the North Atlantic and the biological productivity of the English Channel. The views presented in the Report for 1953–54 have required little change, have been developed further, and are now much more firmly based. All links in the process are now seen clearly, though some are still hypothetical. They are presented schematically in two papers which are to appear in the *Journal of Marine Research* and in the Bigelow Birthday Number of *Deep Sea Research*. Not all the data underlying the arguments have yet been written up; those from the Denmark Strait region are at present being brought together.

Associated hypotheses have been erected for the North Pacific. These are an attempt to relate fluctuations in the success of the fisheries of California and the Pacific coast of Japan with fluctuations in the strength and position of the Eastern Asiatic winter anticyclone. The facts so far unearthed have required no revision of the hypotheses.

The winter maximum for inorganic phosphate at station E1 in 1955 reached a concentration greater than in any of the preceding 24 years. In March and May two cruises to deep water on R.V. *Sarsia* were made by Dr Cooper and Mr Armstrong to find out where this enriched water came from. No clear-cut answer was obtained. Rich water was present along the continental slope between the Great Sole Bank and South-west Ireland, but no connexion of this with the Plymouth area was found. The distribution of properties, especially oxygen, over the continental slope was perplexing. Vigorous water movements were in progress in a highly heterogeneous honeycomb of water masses. With the data available one cannot separate internal wave phenomena, including possible submarine eagres, from along-slope currents. The means are now available to differentiate between the several interpretations which may be placed on the past year's work.

Dr Cooper's detailed account of the occurrence of chemical elements in sea water is being amalgamated with one prepared independently by Dr E. G. Goldberg of the Scripps Institution of Oceanography. An article on 'Oceanography in Past Ages', prepared some years ago for *The Science of Petroleum*, has been brought up to date. A joint paper with Mr J. S. Colman, of Port Erin, on 'Underwater illumination and ecology in tropical estuaries' written in 1939, has now been revised and published in the *Bulletin* of the British Museum (Natural History).

Mr F. A. J. Armstrong has continued the monthly cruises to Station E 1 and analyses for phosphate, total phosphorus and silicate. Further analyses for aluminium have shown that the amount present in solution in sea water is rarely more than about 2 μg atom Al/l. and shows little variation. Some earlier figures indicating larger amounts of aluminium have been rejected, owing to unsuspected contamination by copper from the metal sampling bottles, and the method has been amended to obviate the interference of copper. He has developed a method for the estimation of iron in sea water which appears to determine the total iron content. The results of analysis of samples from E 1 and three deep-water stations show much higher values than have previously been reported, and these are being prepared for publication.

Mr Armstrong has constructed an absorptiometer for colorimetric analysis at sea, and a prototype was tested during a cruise in R.V. *Sarsia*. It appeared to be reliable, and needed little space in the laboratory.

In order to find whether any of the considerable quantity of organic nitrogen, known to be present in solution in sea water, is available as a nitrogen source for the growth of phytoplankton, a method of bioassay of available nitrogen compounds has been developed by Dr H. W. Harvey. The opportunity was taken to compare the quantities found by assay in a water collected in the summer with the quantity of inorganic nitrogen compounds found by analysis by Dr J. P. Riley, of the Department of Oceanography at Liverpool University, using a method which he has developed for the determination of nitrate nitrite and ammonium nitrogen in one operation. The results indicate that almost none of the organic nitrogen was utilized.

Preliminary experiments have been made by Dr Harvey in collaboration with Mr E. Soeriaatmadja, of Indonesia, to investigate the uptake of phosphate at low concentrations in sea water by ferric hydroxide. Treatment with fifty times more iron (as recently precipitated ferric hydroxide) than the phosphate-phosphorus present in sea water resulted in insignificant uptake at pH 8, but considerable uptake at pH 6·5. The uptake and resulting equilibrium appeared to depend upon the ratio of iron to phosphate, their initial concentration, and the pH of the sea water. Kaolin in suspension at a concentration more than a thousand times greater than that of the phosphate caused little change in the phosphate concentration of sea water, but adsorbed all the 10 mg phosphate P per m^3 which had been added to a fresh water.

The first paper by Dr Mary Parke, in collaboration with Prof. Irene Manton and Mr B. Clarke, of Leeds University, on new members of the Chrysophyceae has been published in Vol. 34, No. 3, of the *Journal*. The three species described there are the first of a considerable series of related forms which have been isolated and are being maintained in culture at Plymouth. A further twenty new members of this series have been isolated during the present year. These forms all possess a number of rather unusual characters which make their classification difficult. They have two flagella, in some equal in length and in others unequal, and an additional retractile filiform appendage used for the temporary anchorage of the cell to which the name *haptonema* has been given.

In some species pigmented and non-pigmented individuals have been observed and phagotrophic feeding has already been demonstrated in a large number of the different species. All the species in this series show a covering of sculptured scales, of varied size and shape in different forms; they may or may not bear spines. In the majority the scales are so small that they are invisible under the light microscope and can only be studied successfully under the electron microscope. Prof. Irene Manton is continuing to collaborate with Dr Parke on this side of the work. The study of another group of these new forms should be completed by the end of the year.

The collection of unialgal cultures of marine phytoplankton organisms has been maintained throughout the year, with the assistance of Miss I. M. Adams. A very large number of cultures have been distributed for research purposes to institutions in this country and abroad.

Miss D. Ballantine has continued her experiments on the toxicity of flagellates in particular on the toxin from *Gymnodinium veneficum* nom. prov. This work has been done in collaboration with Dr Abbott. The toxin has been isolated from cultures by adsorption on active charcoal followed by elution with organic solvents. It is believed that a reasonably pure extract has been obtained, and this is being used to study its physiological effects on animals and isolated tissues. It renders muscle inexcitable to either direct or indirect stimulation, and reduces both action and membrane potentials in muscle and in certain nerves. Samples of the toxin have also been sent to Dr J. M. Ritchie, of the National Institute of Medical Research.

The results of Miss Ballantine's work with Dr J. E. Morton of Queen Mary College, London, on *Lasaea rubra* were published in Vol. 35, No. 1 of the *Journal*; a filtering rate of 0·05 ml./h/animal was obtained, using acceptable organisms, but filtering can be reduced or stopped when toxic flagellates are used. The distinction between filtering and feeding has been made, and it is found that in algal cultures *Lasaea* is capable of filtering off many more organisms than it can ingest. This probably does not occur in nature. It is proposed to follow this up with an investigation of the digestive system of *Mytilus*.

Some work has been done on the sand-living dinoflagellates at Port Erin. It is hoped to continue this work, and to examine specimens from this and other areas.

Mr F. S. Russell has been examining collections of deep-sea jellyfish made on the research vessel *Sarsia*. Some interesting Scyphomedusae have been found, among which were numerous well-preserved specimens of a new species of the extremely rare genus *Paraphyllina*. A description of this species has been published in Vol. 35, No. 1, of the *Journal*. It is hoped also to describe certain species of the genus *Nausithoë*; and two Hydromedusae which appear to be new species.

Mr P. G. Corbin's examination of the 1955 catches of the 2 m stramin ring-trawl showed that *Sagitta setosa* continues to prevail and the level of macro-plankton production to remain low.

During the 1955 breeding season of *Echinus esculentus* Dr D. P. Wilson and Mr Armstrong continued their experiments on biological differences between sea waters. Once again they were hampered by weather and other factors beyond their control, but none the less made some progress. This year the difference between Clyde and E1 waters was more marked than in the immediately preceding years and was nearly as great as during the early experiments. It was again found that the *Echinus* eggs and larvae behaved differently in bottom and surface waters collected at the same time and at the same station. Several water samples were successfully filtered free from bacteria at the time of collection, and these waters showed only insignificant differences from those collected in the ordinary way. Unfortunately, the experiments were to some extent vitiated by the poor condition of the *Echinus* eggs. Indeed adult *Echinus* were unusually scarce on the usual trawling grounds, owing perhaps to severe gales earlier in the year. Other results included some with artificial sea water, using both *Echinus* larvae and larvae of *Sabellaria alveolata*. The results are being written up for publication in the *Journal*.

Macro-Fauna and Flora

Dr D. P. Wilson's experiments, made during 1954, on the settlement of *Ophelia bicornis* and referred to in the last report, have during the current year been fully considered, prepared for publication and published in Vol. 34, No. 3, of the *Journal*. The preliminary assessment already reported was found to be substantiated, and this work has therefore reached a stage where further development is not immediately possible. The next phase would appear to involve identification of the bacteria and other micro-organisms occurring on the sand grains of the natural adult habitat, their growth in pure cultures and the testing of their influence singly and mixed after transfer to acid-cleaned sands. Until this can be done progress is halted.

Collections made in the Helford River in 1949 by Mr G. M. Spooner and Mr Holme have been fully worked out, and the results have been supplemented by fresh collections made this year by Mr Spooner on the east side of the Fal Estuary, chiefly by digging and surface collecting. The seaward ends of the estuaries in this part of Cornwall contain a rich fauna, about which our knowledge has been incomplete. Interesting comparisons with the more restricted grounds at Salcombe and Noss Mayo can be made. One unexpected find was *Myxicola infundibulum* flourishing in clean sand, and a populous bed of *Labidoplax digitata*.

Mr Spooner has also studied the three British species of the Clunioninae (an aberrant sub-family of Chironomidae), particularly with a view to determining the characters of the larvae and to obtain a clearer picture of their distribution on the shore. The larva of each species proves, happily, to possess diagnostic features by which it can be separated from the other two and from any orthocladine gnat. *Psammathiomyia pectinata*, a lusitanian species of exposed rocky shores, seldom hitherto observed, was discovered in two new localities on the north coast of Cornwall. Emergences of adults are evidently very sporadic, and the duration of adult life is found to be only 1 or 2 h. The larvae, now observed and recognized for the first time, will provide the best means of detecting the species on the shore.

The larvae of *Clunio marinus* are frequent amongst algal collections from rocks in the Plymouth area, and the species is evidently more general than would be judged by observation of adults. *Thalassomyia frauenfeldi* appears to be restricted to *Enteromorpha* patches in the upper half of the tidal zone: adults emerge throughout the winter, and can be found on sheltered rocks in the stormiest or coldest period.

Specimens of the *Gobius* sp., collected by Mr G. R. Forster while diving off Rame Head, have been examined by Mr Corbin. The species is certainly new to the British list and very probably new to the European list. Mr Corbin has also been collecting data of another *Gobius* sp. close in appearance to the Rock Goby, *G. paganellus*, but distinct in several characteristics, particularly its off-shore habitat, where it is not infrequently taken in the Agassiz trawl or fine mesh cover of the otter trawl. It is not recorded in the British list, but may possibly prove to be among the recorded European species.

Mr Corbin has recently been examining the hermit crabs taken in Agassiz trawl hauls in order to assess the relative abundance of the Plymouth species. *Anapagurus hyndmanni*—unrecorded since 1912—is, together with *A. laevis*, one of the most abundant species: 150–200 of each may be caught in a 15 min haul. Small *Eupagurus prideauxi* are about half as numerous. *E. cuanensis* is caught regularly in small numbers—under half a dozen. Small *E. bernhardus* are even less numerous off-shore in contrast to their intertidal abundance in periwinkle shells. *E. sculptimanus* is the least common of the listed species: five specimens were found in 1955. One specimen of *Catapaguroides timidus*, previously unrecorded, was taken. The foraminiferan, *Astorhiza limicola*, was also found in the Agassiz hauls: it is also unrecorded for the area. It occurs in fair numbers and is of considerable size, up to 1·5 cm diameter, and may thus well be an important food organism for bottom feeders.

Mr N. A. Holme has continued work on the ecology of lamellibranchs, particular attention being paid to the distribution of certain species southward from the British Isles. In April 1955 a week was spent shore-collecting on sandy beaches in S. Brittany, most of the collections being from sheltered shores in Quiberon Bay. The fauna here is little different from that in south-west England, but the abundance of the lusitanian species *Sipunculus nudus* and the relatively higher level at which low-tide species occurred on the shore were noted. In June a cruise on R.V. *Sarsia*, using the bottom-sampler, was devoted to a study of the off-shore fauna of the area. Samples were taken in the Grande Vasière and in shallow water inside Belle Ile, in Quiberon Bay, and off Lorient. All these collections will be used for a general study of the distribution of lamellibranchs, with special reference to particle size of the soil.

Further trials of the new 'vacuum' grab have been made, and the instrument has been tested to a depth of 448 m with success. Some modifications to the original grab have been made to aid vertical descent through the water. A description of the grab appeared in Vol. 34, No. 3, of the *Journal*.

Mr G. R. Forster has completed notes on the sponge *Hemimycale columella*, and on the differences between the sublittoral rock fauna of Plymouth and Dartmouth, and these have been published in Vol. 34, Nos. 2 and 3, of the *Journal*.

In 1955 the underwater collecting from the yacht *Sunset* has been continued, thirty-nine dives having been made from July till October, including five near St Helier, Jersey, and six in Falmouth Bay. Further samples of sessile animals have been obtained, and in addition several living specimens of an unusual goby, previously referred to in this report, which is conspicuously marked by large red or brown spots.

In April Mr Forster spent a week shore-collecting from the Station Biologique de Roscoff, and much help was received in the identification of sponges. One sponge, *Stelletta grubei*, well known at Roscoff, has since been taken on the Eddystone reef.

An annual population sample of young prawns, *Palaemon* (= *Leander*) *serratus*, has been taken each year since 1949, to ascertain whether the growth of the young O group prawns is related to variations in temperature. From July to October 1955, the mean of the daily sea-surface temperatures was 2·0° C higher than that of 1954, but the mean length of the O group prawns showed only a very slight increase over the previous year.

Dr A. J. Southward, on a D.S.I.R. research grant, is studying the distribution, breeding and ecology of intertidal animals. Regular observations on the barnacle populations around Plymouth, and elsewhere in Britain, have made it clear that, following a recession up to 1951–52, the northern species, *Balanus balanoides*, is now returning in some numbers to the south-west of England. The recent fluctuations in the distribution and abundance of this barnacle, and of the southern species *Chthamalus stellatus*, are discussed in a contribution to the *Journal*, Vol. 35, No. 1, written in collaboration with Dr D. J. Crisp of the Marine Biology Station, Bangor. Dr Southward and Dr Crisp are also preparing a series of papers on the distribution of the British intertidal fauna;

the first will deal with the English Channel coasts. In collaboration with Dr J. M. Dodd of St Andrews University, Dr Southward is preparing for publication the late Prof. J. H. Orton's work on the biology and breeding of British limpets. An introductory note, and a description of the breeding of the common limpet have been contributed to the *Journal*.

With his wife, Dr Eve C. Southward, Dr Southward is investigating the breeding of the lugworms *Arenicola ecaudata* and *A. branchialis*, which live on the shore in muddy gravel under stones. Specimens containing gametes can be found all the year round, and the spawning periods appear to be longer than in the sand-living *A. marina*. So far, there seems little relation between the geographical distribution of these animals and their breeding periods.

Dr Southward and Mr Forster are studying the biology of the barnacle *Pyrgoma anglicum*, which grows on living cup-corals. The barnacle shell is intimately mingled with the coral skeleton, and is covered by a layer of coral tissue except round the aperture. It is hoped to find out the nature of the relationship between the two animals, and to work out the breeding and settlement of the barnacle.

Dr Southward visited the Isles of Scilly during his distributional work. As the marine fauna of these Islands is not fully known, some general faunistic surveys were made with Dr Eve C. Southward. It was discovered or confirmed that several intertidal animals common in Britain are absent or very rare in the Isles of Scilly. These Islands are also interesting for the occurrence there intertidally of some animals found otherwise only sublittorally. A preliminary fauna list is in preparation, and it is hoped to make further visits in collaboration with Mr Forster.

Dr Southward is also investigating the physiology and behaviour of barnacles. Papers on the frequency of cirral beat, and the effects on it of temperature and habitat have been published in Vol. 34, No. 3, of the *Journal*.

Preliminary experiments have shown that barnacles can feed on a wide range of organisms, from 1 mm down to 2 μ in length, and it is proposed to compare the rate of feeding, and respiration, of the different species. The cirral mechanisms, and the reactions of the cirri to water movements are being studied cinematographically in collaboration with Dr D. J. Crisp.

Physiology of Marine Organisms

Utilizing the luminescent response as an indicator of neuro-effector activity, Dr J. A. C. Nicol has continued his researches into neural functioning among invertebrates. Some of the earlier physiological studies of luminescence were made on sea pens (Pennatulacea), and it has now become possible to secure quantitative information about the luminescent responses of these animals by the use of photo-electric instruments. Parameters of the luminescent response and conduction velocity in the nerve net have been measured, and analyses made of neuro-neural and neuro-photocyte transmission (*Renilla*, *Leioptilus* and *Pennatula*). An account of part of this work has appeared in the *Journal of Experimental Biology*, Vol. 32, p. 299 (1955). Particular attention is now being paid to investigating the responses of single zooids. Another paper, in collaboration with Dr D. Davenport, in the *Proceedings of the Royal Society*, Vol. 144, describes the luminescent responses of Hydromedusae, especially *Aequorea*. Light is emitted by patches of photogenic tissue in the marginal canal, and appears as brief flashes, *ca.* 1 sec in duration. Although no evidence was obtained for propagation of luminescent waves, it was found that the intensity of the separate flashes was influenced by the character of stimuli, in the same manner as in the neuromuscular system of other coelenterates. Dr Nicol has also contributed a review paper to a symposium on luminescence, which has appeared in *The Luminescence of Biological Systems*, p. 299, published by the American Association for the Advancement of Science, 1955.

Dr J. S. Alexandrowicz has completed his study on receptor elements in the muscles of *Leander serratus*. In the 1st to 5th abdominal segments two muscle receptor organs have been found on each side. An unusual arrangement is shown in the median receptors in the 1st and 2nd segments in which the two nerve cells are connected with one long receptor muscle running through two segments. In the thorax only two receptor muscles have been found, one of which connects with four nerve cells. It is suggested that the three supernumerary cells may be remnants of the retrograding receptor organs in the thoracic segments which are fusing together and losing their motility. Receptor elements of a different category, the N-cells, have been found, five in number, sending their dendritic expansions into one of the thoracico-abdominal muscles. It has been established that their axons join the nerve trunks of the 3rd–7th thoracic segments. The results are being published in the *Journal*.

A further part of the 'Notes on the nervous system in the Stomatopoda', dealing with the sensory elements of *Squilla mantis*, has been prepared for publication. It has been found that in these animals, besides sensory cells of the common type, i.e. with short single distal processes, there are cells with very long branching processes spreading their terminal ramifications under the epithelium of the integument. A description of these elements and a survey of various types of sensory nerve cells in stomatopods is being published in *Pubblicazioni della Stazione Zoologica di Napoli*.

Dr D. B. Carlisle has continued work on the endocrinology of crustaceans, particularly on a water-balance or antidiuretic hormone which he has isolated in *Carcinus*. This hormone, emanating from the X organ–sinus gland complex, plays a part in restraining the amount of size increase at each moult and in osmo-regulation, besides its antidiuretic function. It is not one of the two known moulting hormones. In collaboration with Dr D. Lacy of the Department of Zoology, St Bartholomew's Medical College, an investigation has begun into the ultracytology of the 'kidney' of *Carcinus* and into the changes which it undergoes upon hormonal stimulation.

Dr Carlisle's collaboration with Mme M. Dupont-Raabe of the Sorbonne, Paris, and Sir Francis Knowles of Marlborough into the endocrinology of colour change in *Leander* and the stick-insect *Carausius*, has been most profitable and has led to the separation and partial characterization of a number of chromactivating substances: this work is continuing. A preliminary account has been published in *C.R. Acad. Sci., Paris*, and a longer paper presenting some of the results has appeared in Vol. 34, No. 3, of the *Journal*.

The physiological evidence of differences in the populations of *Leander* from Plymouth and Roscoff, reported last year, have been further confirmed, and morphological differences have been found also. An account has appeared in Vol. 34, No. 3, of the *Journal*.

Dr Carlisle, in collaboration with Dr C. G. Butler of the Bee Department, Rothamsted Agricultural Research Laboratory, has begun an investigation of the ovarian inhibiting hormone of prawns and the 'queen-substance' of bees. These substances appear, from the preliminary experiments, to be similar in nature and function, and may possibly even be identical. A preliminary account has been published in *Nature*.

In collaboration with Dr Lacy and Dr A. J. Southward, Dr Carlisle has begun an investigation into the micro-anatomy, cytology, and possible function of a presumed endocrine organ in the pedal ganglion of *Patella*.

Dr Carlisle has continued his work on the Plymouth tunicates, concentrating especially on an investigation of the locomotory powers of adult ascidians.

Dr B. C. Abbott has continued to study the uptake from the sea of certain radioactive products of uranium fission. Experiments on the radioactive yttrium have been concluded: the uptake of algae is by adsorption and is accentuated by the tendency of yttrium to form radiocolloids and to precipitate. Attention has also been concentrated on the fission product Niobium 95. In conjunction with Dr Carlisle the uptake of niobium has been compared with that of radio-vanadium (obtained by courtesy of the Physics Department of Birmingham University), both by cellophane surfaces and by several species of ascidians.

Measurements of the thermal and mechanical properties of lamellibranch muscles have been continued, partly in conjunction with Dr J. Lowy of Queen's University, Belfast. Heat production has been demonstrated during the long maintained contractions induced in the *Mytilus* anterior byssus retractor muscle by the addition of acetylcholine. Isolated muscle and nerve-muscle preparations from other lamellibranchs are under review.

Thermal measurements are also being carried out on nerve tissue under the guidance of Prof. A. V. Hill, and in conjunction with Mr J. V. Howarth of University College, London. The initial heat production in the motor nerves of crabs, associated with brief tetanic stimulation, has been measured with thermopile and fast, sensitive recording equipment.

Dr E. D. S. Corner, International Paints Research Fellow, is making an investigation of the effects of heavy metal poisons on crustaceans.

Preliminary studies have been concerned with the changes which occur in the respiration rate and motility of *Artemia salina* larvae when these animals are placed in sea water containing copper, mercury, and other poisons. The results so far obtained indicate that observed differences between the toxicities of various compounds of mercury (e.g. mercuric chloride, mercuric iodide and ethylmercury chloride) may be attributed to differences in the ease with which the respective substances penetrate the animal to produce their effects internally. There are also indications that the mode of action of copper may differ from that of mercury.

By courtesy of Mr O. D. Hunt, experiments have also been conducted in the International Paints Research Laboratory at Newton Ferrers. In collaboration with Mr B. W. Sparrow, a study has been made of the effects of various organomercurial compounds, and of other poisons on the copepod *Acartia clausi*. The results of this investigation are consistent with the view that the mercury compounds so far studied act by penetrating the animal and inhibiting metabolic changes.

It is hoped to extend these studies by testing different compounds of mercury, and of copper, as inhibitors of isolated enzymes and enzyme systems.

Before he left the staff in September, Dr H. G. Vevers was able to continue his work on the pigmentation of marine invertebrates. In addition to work on the carotenoid pigments of certain echinoderms, he has continued his collaboration with Dr G. Y. Kennedy, of the University of Sheffield, on the occurrences of porphyrins in marine animals. They have prepared a paper on the presence of uroporphyrin I in the tectibranch mollusc *Akera bullata*, which has been published in Vol. 35, No. 1, of the *Journal*.

The Library

The thanks of the Association are again due to many foreign Government Departments, to Universities and to other Institutions at home and abroad for copies of books and current numbers of periodicals either presented to the Library or received in exchange for the *Journal* of the Association.

Thanks are also due to those who have sent books or reprints of their papers, which are much appreciated.

The Library has again been much used by visiting members of the Association.

The new library extension has been in use during the year. Miss L. M. Serpell, with the assistance of Dr J. A. C. Nicol, has been much occupied in planning the reclassification and rearrangement of the books and periodicals.

Published Memoirs

Volume 34, No. 2, of the *Journal* was published in June 1955, No. 3 in October 1955 and Volume 35, No. 1, in February 1956.

The following papers, the outcome of work done at the Plymouth laboratory, have been published elsewhere than in the *Journal* of the Association:

Abbott, B. C., 1955. Biophysics in marine biology. *Sci. Progr.*, Vol. 43, pp. 629–33.

Atkins, D., 1955. *Pythium thalassium* sp. nov. infecting the egg-mass of the pea-crab, *Pinnotheres pisum*. *Trans. Brit. mycol. Soc.*, Vol. 38, pp. 31–46.

Atkins, W. R. G. & Jenkins, Pamela G., 1955. Identification of water-masses by their suspended matter. *Nature*, Vol. 175, p. 951.

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Membership of the Association

The total number of members on 31 March 1956 was 861, being 34 more than on 31 March 1955; of these the number of life members was 98 and of annual members 763. The number of associate members is five.

Finance

General Fund. The thanks of the Council are again due to the Development Commissioners for their continued support of the general work of the laboratory.

Private Income. The Council gratefully acknowledge the following generous grants received during the year:

From the Fishmongers' Company (£400), British Association (£50), Physiological Society (£30), the Cornwall Sea Fisheries Committee (£10), the Universities of London (£210), Cambridge (£125), Oxford (£100), Bristol (£50), Birmingham (£31. 10*s*.), Leeds (£20), Durham (£10. 10*s*.), Manchester (£10. 10*s*.), Sheffield (£10. 10*s*.), Southampton (£15. 15*s*.), Reading (£10. 10*s*.), Nottingham (£10. 10*s*.), Hull (£10. 10*s*.), Exeter (£10. 10*s*.), Leicester (£10. 10*s*.), the Imperial College of Science and Technology (£10) and the Zoological Society of London (£10. 10*s*.).

President, Vice-Presidents, Officers and Council

The following is the list of those proposed by the Council for election for the year 1956–57:

President

Prof. A. V. HILL, C.H., O.B.E., Sc.D., LL.D., F.R.S.

Vice-Presidents

The Earl of IVEAGH, K.G., C.B., C.M.G.
Sir NICHOLAS E. WATERHOUSE, K.B.E.
Col. Sir EDWARD T. PEEL, K.B.E., D.S.O., M.C.
Vice-Admiral Sir JOHN A. EDGELL, K.B.E., C.B., F.R.S.
Sir EDWARD J. SALISBURY, Kt., C.B.E., D.Sc., F.R.S.
Admiral Sir AUBREY C. H. SMITH, K.B.E., C.B., M.V.O.
A. T. A. DOBSON, C.B., C.V.O., C.B.E.
Major E. G. CHRISTIE-MILLER
MORLEY H. NEALE, C.B.E.
The Earl of VERULAM
Prof. Sir JAMES GRAY, Kt., C.B.E., M.C., Sc.D., LL.D., F.R.S.

COUNCIL

To retire in 1957

Miss ANNA M. BIDDER, Ph.D.
D. J. CRISP, Ph.D.
Prof. J. E. HARRIS, Ph.D., F.R.S.
C. E. LUCAS, C.M.G., D.Sc.
Prof. C. M. YONGE, C.B.E., D.Sc., F.R.S.

To retire in 1958

Miss VERA FRETTER, D.Sc.
MICHAEL GRAHAM, C.M.G., O.B.E.
N. A. MACKINTOSH, C.B.E., D.Sc.
G. E. NEWELL, Ph.D.
Prof. LILY NEWTON, D.Sc.

To retire in 1959

G. E. R. DEACON, C.B.E., D.Sc., F.R.S.
M. N. HILL, Ph.D.
O. D. HUNT
Prof. R. J. PUMPHREY, Sc.D., F.R.S.
Prof. G. P. WELLS, Sc.D., F.R.S.

Hon. Treasurer

Major E. G. CHRISTIE-MILLER, 38 Hyde Park Street, London, W. 2.

Secretary

F. S. RUSSELL, C.B.E., D.S.C., D.F.C., F.R.S.
The Laboratory, Citadel Hill, Plymouth

The following Governors are also members of the Council:

R. G. R. WALL (Ministry of Agriculture, Fisheries and Food)
The Worshipful Company of Fishmongers:
  The Prime Warden
  Major E. G. CHRISTIE-MILLER
  HARRISON S. EDWARDS
Prof. A. C. HARDY, D.Sc., F.R.S. (Oxford University)
S. SMITH, Ph.D. (Cambridge University)
EDWARD HINDLE, Sc.D., F.R.S. (British Association)
N. B. MARSHALL (Zoological Society)
Prof. Sir JAMES GRAY, Kt., C.B.E., M.C., Sc.D., LL.D., F.R.S. (Royal Society)

BALANCE SHEET 1955–56

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

BALANCE SHEET 31 MARCH 1956

	£	£
CAPITAL RESERVE ACCOUNT:		
As at 31st March 1955	170,972	
Add: Expenditure on fixed assets recovered	3,855	174,827
SURPLUS ACCOUNT:		
As at 31st March 1955	8,146	
Add: Excess of income over expenditure for the year	754	
	8,900	
Less: Provision for diminution in value of investment	44	8,856
		183,683
BALANCES ON SPECIAL FUNDS (see annexed statement)	9,009	
Less: Provision for diminution in value of investments	1,487	7,522
CURRENT LIABILITIES:		
Sundry creditors and accrued expenses	1,738	
Subscriptions and grants received in advance	151	
Equipment and apparatus—R.V. 'Sarsia'	—	1,889
		£193,094

Note: Capital commitments outstanding amount to £10,193 (1955 £8,522) of which £7,555 will be recoverable under Development Fund grants.

D. J. CRISP
EDWARD HINDLE } *Members of the Council*

	£		£
FIXED ASSETS:			
Boats and Equipment:			
At cost:			
R.V. 'Sarsia'	137,761		
At valuation as estimated by the Director at 31st March 1956:			
M.F.V. 'Sula'	12,500		
R.L. 'Gammarus'	200	150,461	
Laboratory apparatus, equipment and machinery:			
At cost	13,380		
Less: Depreciation	1,338	12,042	
Library at valuation of Mr Ridgill Trout in January 1941 plus additions at cost		21,600	184,103
INVESTMENTS:			
General Fund at book amount (Market value £188; last year £217)		232	
Composition Fees Fund at cost (Market value £1,031; last year £1,110)		1,330	
E. T. Browne Bequest Funds at cost (Market value £3,180; last year £3,440)		4,368	
		5,930	
Less: Provision for diminution in value of investments		1,531	4,399
CURRENT ASSETS:			
Stocks on hand as valued by the Director		1,550	
Sundry debtors		885	
Prepayments		211	
Balances at bank and cash in hand		1,946	4,592
			£193,094

REPORT OF THE AUDITORS TO THE MEMBERS OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM:

Capital expenditure on the erection of buildings on land held on lease from the War Department is excluded. Subject to the foregoing, in our opinion and to the best of our information and according to the explanations given to us, the above balance sheet and annexed income and expenditure account give a true and fair view of the state of the Association's affairs as at 31st March 1956, and of the excess of income over expenditure for the year ended on that date.

We have obtained all the information and explanations which to the best of our knowledge and belief were necessary for our audit. In our opinion the Association has kept proper books of account and the above mentioned accounts, which are in agreement therewith, give in the prescribed manner the information required by the Companies Act, 1948.

PRICE WATERHOUSE & CO.
Chartered Accountants

Norwich Union House
2 St Andrew's Cross
Plymouth
17 *May* 1956

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31st MARCH 1956

	£	£
SALARIES, NATIONAL INSURANCE AND SUPERANNUATION SCHEME CONTRIBUTIONS		27,010
LABORATORY AND BOATS' CREWS' WAGES, NATIONAL INSURANCE, SUPERANNUATION SCHEME CONTRIBUTIONS AND EMPLOYERS' LIABILITY INSURANCE		26,506
UPKEEP OF LIBRARY		699
SCIENTIFIC PUBLICATIONS, *less* SALES		1,666
UPKEEP OF LABORATORIES:		
Buildings and machinery	609	
Electricity, gas, coal and water	1,317	
Chemicals and apparatus	1,457	
Depreciation of Laboratory apparatus, equipment and machinery (no depreciation is provided on the other fixed assets)	1,338	
Rents and insurance	175	
Travelling expenses	685	
Audit fee (1955 includes £63 for previous year)	126	
Stationery, postage, telephone and sundries	1,235	
Specimens	195	
Collecting expenses and upkeep of truck	298	
		7,435
MAINTENANCE OF BOATS:		
Petrol, oil, paraffin etc.	1,721	
Maintenance and repairs	3,641	
Insurance	2,366	
Hire of Decca Navigator—R.V. 'Sarsia'	395	
		8,123
ENTERTAINMENT EXPENSES		54
BALANCE being excess of income over expenditure for the year		754
		£72,247

	£	£	£
GRANTS AND TABLE RENTS:			
Ministry of Agriculture, Fisheries and Food Grant from Development Fund		64,970	
Fishmongers' Company		400	
Miscellaneous (including British Association £50, Royal Society £50, Physiological Society £30, Cornwall Sea Fisheries Committee £10, Universities of London £210, Cambridge £125, Oxford £100, Bristol £50, Birmingham £31. 10*s.*, Leeds £20, Southampton £15. 15*s.*, Durham £10. 10*s.*, Exeter £10. 10*s.*, Leicester £10. 10*s.*, Manchester £10. 10*s.*, Nottingham £10. 10*s.* Hull £10. 10*s.*, Reading £10. 10*s.* and Sheffield £10. 10*s.*, Imperial College £10, Zoological Society of London £10. 10*s.*, Ministry of Works £104, Imperial Chemical Industries Ltd. £52. 10*s.* and International Paints Ltd. £52. 10*s.*)		1,230	
			66,600
SUBSCRIPTIONS (excluding those received in advance)			777
SALES:			
Specimens		2,882	
Fish		705	
Nets, gear and hydrographical apparatus	825		
Less: cost of materials	587		
		238	
			3,825
SUNDRY PUBLICATIONS			18
INCOME FROM INVESTMENTS			49
INTEREST ON BANK DEPOSITS, *less* CHARGES			75
AQUARIUM:			
Admission fees		1,414	
Sale of guides		89	
		1,503	
Less: Maintenance, printing and advertising		600	
			903
			£72,247

MOVEMENTS ON SPECIAL FUNDS DURING THE YEAR TO 31st MARCH 1956

			E. T. Browne Bequest											
	Aquarium Sinking Fund	Com-position Fees	Library	Special Apparatus	Scientific Publications	A. R. T. Momber Bequest	Rockefeller Foundation Fund	War Damage Com-pensation Part II	Library Extension and Dogfish House	'Plymouth Marine Fauna' Fund	M.F.V. 'Sula' Winch	Reservoir and Sea Water Tanks	Research Funds*	TOTAL
	£	£	£	£	£	£	£	£	£	£	£	£	£	£
BALANCE AT 31ST MARCH 1955	341	1,251	1,335	2,876	(21)	1,090	518	45	1,567	—	—	—	1,212	10,214
Add: Income during year:														
Grants	—	—	—	—	—	—	2,680	—	4,375	750	2,000	1,885	2,429	14,119
Income from investments	—	—	41	83	7	—	—	—	—	—	—	—	—	131
Bank deposit interest	10	—	—	—	1	34	—	—	—	—	—	—	—	45
Other income	35	78	—	—	377	—	—	—	—	—	—	—	—	490
Transfers-Income and Expenditure account	—	—	—	—	—	—	—	—	—	—	5	—	5	10
	386	1,329	1,376	2,959	364	1,124	3,198	45	5,942	750	2,005	1,885	3,646	25,009
Deduct: Expenditure	—	—	—	60	—	—	3,207	45	4,589	753	2,005	1,885	3,456	16,000
BALANCE AT 31ST MARCH 1956	£386	£1,329	£1,376	£2,899	£364	£1,124	(£9)	—	£1,353	(£3)	—	—	£190	£9,009

* Including International Paints Ltd. Research Fellowship.

LIST OF GOVERNORS, FOUNDERS, MEMBERS, HONORARY AND ASSOCIATE MEMBERS

1956

GOVERNORS

THE BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE, Burlington House, W. 1
THE UNIVERSITY OF OXFORD
THE UNIVERSITY OF CAMBRIDGE
THE WORSHIPFUL COMPANY OF CLOTHWORKERS, 48 Fenchurch Street, E.C. 3
THE WORSHIPFUL COMPANY OF FISHMONGERS, London Bridge, E.C. 4
  THE PRIME WARDEN. (**Council**, 1886→)
  EDWARDS, HARRISON S., Westhumble Lacey, near Dorking, Surrey. (**Council**, 1950→; **Hon. Treasurer**, 1956→)
  CHRISTIE-MILLER, Major E. G., 38 Hyde Park Street, W. 2. (**Council**, 1941→; **Hon. Treasurer**, 1941→56; **Vice-President**, 1951→)
THE ZOOLOGICAL SOCIETY OF LONDON, Regent's Park, N.W. 1
THE ROYAL SOCIETY, Burlington House, Piccadilly, W. 1
MINISTRY OF AGRICULTURE, FISHERIES & FOOD, 3 Whitehall Place, S.W. 1
BAYLY, ROBERT (the late). (**Council**, 1896–1901)
BAYLY, JOHN (the late)
BROWNE, E. T. (the late). (**Council**, 1913–19; 1920–37)
THOMASSON, J. P. (the late). (**Council**, 1896–1903)
BIDDER, G. P., Sc.D. (the late). (**Council**, 1899–1953; **President**, 1939–45; **Vice-President**, 1948–53)
THE Lord MOYNE, P.C., D.S.O. (the late). (**Vice-President**, 1929; 1939–45; **President**, 1930–39)
ALLEN, E. J., C.B.E., D.Sc., LL.D., F.R.S. (the late) (Honorary). (**Council**, 1895–1942; **Secretary**, 1895–1936; **Hon. Governor**, 1937–42)

FOUNDERS

1884 THE CORPORATION OF THE CITY OF LONDON, The Guildhall, E.C. 3
1884 THE WORSHIPFUL COMPANY OF MERCERS, Mercers' Hall, 4 Ironmonger Lane, E.C. 2
1884 THE WORSHIPFUL COMPANY OF GOLDSMITHS, Goldsmiths' Hall, Foster Lane, E.C. 2
1884 THE ROYAL MICROSCOPICAL SOCIETY, B.M.A. House, Tavistock Square, W.C. 1
1884 BULTEEL, THOS. (the late)
1884 BURDETT-COUTTS, W. L. A. BARTLETT (the late)
1884 CRISP, Sir FRANK, Bart. (the late). (**Council**, 1884–92; **Hon. Treasurer**, 1884–88)
1884 DAUBENY, Captain GILES A. (the late)
1884 EDDY, J. RAY (the late)
1884 GASSIOTT, JOHN P. (the late)
1884 LANKESTER, Sir E. RAY, K.C.B., F.R.S. (the late). (**Hon. Secretary**, 1884–90; **President**, 1891–1929)

1884 Lord MASHAM (the late)
1884 MOSELEY, Prof. H. N., F.R.S. (the late). (**Chairman of Council**, 1884–88)
1884 Lord AVEBURY, F.R.S. (the late). (**Vice-President**, 1884–1913)
1884 POULTON, Prof. Sir EDWARD B., F.R.S. (the late). (**Council**, 1888–94)
1884 ROMANES, Prof. G. J., LL.D., F.R.S. (the late). (**Council**, 1884–91)
1884 WORTHINGTON, JAMES (the late)
1885 The 15th EARL OF DERBY (the late)
1887 WELDON, Prof. W. F. R., F.R.S. (the late). (**Council**, 1890–1901; representing British Association, 1901–5)
1888 BURY, HENRY, The Gate House, 17 Alumdale Road, Bournemouth West
1888 THE WORSHIPFUL COMPANY OF DRAPERS, Drapers' Hall, E.C. 2
1889 THE WORSHIPFUL COMPANY OF GROCERS, Grocers' Hall, Princes Street, E.C. 2
1889 THOMPSON, Sir HENRY, Bart. (the late). (**Vice-President**, 1890–1903)
1889 Lord REVELSTOKE (the late)
1890 RICHES, T. H. (the late). (**Council**, 1920–25)
1892 BROWNE, Mrs E. T. (the late)
1898 WORTH, R. HANSFORD, M.Inst.C.E. (the late)
1899 The EARL OF IVEAGH, K.G., C.B., C.M.G., 11 St James's Square, S.W. 1 (**Vice-President**, 1929→)
1902 GURNEY, ROBERT, D.Sc. (the late). (**Council**, 1932–5)
1904 SHAW, JOSEPH, K.C. (the late)
1909 HARDING, Colonel W. (the late)
1910 MURRAY, Sir JOHN, K.C.B., F.R.S. (the late). (**Council**, 1896–99; **Vice-President**, 1900–13)
1912 SWITHINBANK, H. (the late)
1913 SHEARER, Dr CRESSWELL, F.R.S. (the late)
1913 HERON-ALLEN, E., F.R.S. (the late)
1918 EVANS, GEORGE (the late). (**Hon. Treasurer**, 1915–31; **Vice-President**, 1925–33)
1920 MCCLEAN, Capt. W. N., 39 Phillimore Gardens, W. 8
1920 Lord BUCKLAND OF BWLCH (the late)
1920 LLEWELLYN, Sir D. R. (the late)
1921 HARMER, F. W. (the late)
1924 THE MACFISHERIES, LTD., Ocean House, Pudding Lane, E.C. 3
1924 Lady MURRAY (the late)
1925 THE INSTITUTION OF CIVIL ENGINEERS, Great George Street, Westminster, S.W. 1
1925 DISCOVERY COMMITTEE
1927 BIDDER, Miss ANNA M., Ph.D., 6A Hills Avenue, Cambridge. (**Council**, 1948–51, 1954→)
1933 PEEL, Col. Sir EDWARD T., K.B.E., D.S.O., M.C., c/o Messrs Peel and Co., Ltd. P.O. Box 331, Alexandria, Egypt. (**Vice-President**, 1936→)
1938 BUCHANAN, Dr FLORENCE (the late)
1945 BROWN, ARTHUR W. W. (the late)

MEMBERS

* Life Members

1949 ABBOTT, B. C., Ph.D., F.Inst.P., The Laboratory, Citadel Hill, Plymouth, Devon
1945 ABERDEEN UNIVERSITY LIBRARY, The University, Aberdeen
1934 ADAM, Mrs K. M. G., 84 Lasswade Road, Edinburgh 9

1951 Adams, E., 2 Woodford Crescent, Marsh Mills, Plympton, Devon
*1954 Adams, Miss M. N. E., 61 Ethelburt Avenue, Swaythling, Southampton.
1940 Lord Adrian, O.M., M.D., D.Sc., LL.D., F.R.S., The Master's Lodge, Trinity College, Cambridge
1947 Affleck, R. J., 1 Helmsdale Road, London, S.W. 16
1950 Alexandrowicz, J. S., Ph.D., M.D., The Laboratory, Citadel Hill, Plymouth, Devon
1954 Allen, G. L., The Nook, 87A Bury Old Road, Sedgley Park, Prestwich, Manchester
1951 Allen, J. A., Dove Marine Laboratory, Cullercoats, Northumberland
1952 Allen, Miss J. M., Tenements Farm, Chipperfield, Herts
1953 Alvariño, Señora A., Doce de Octubre 11–1 °C, Madrid, Spain
*1927 Amirthalingam, C., Ph.D., 2 Dickmans Path, Colombo, Ceylon
1956 Ansell, A. D., 22 Mannion Road, Henley-on-Thames, Oxon
1950 Arnold, D. C., Gatty Marine Laboratory, The University, St Andrews, Fife
1944 Ashby, D. G., Littlecroft, High Street, Harston, Cambs
1954 Ashhurst, Miss D. E., Heron Court, Alexandra Road, Epsom, Surrey
*1929 Atkins, Miss D., D.Sc., c/o The Laboratory, Citadel Hill, Plymouth, Devon
*1939 Atkins, W. R. G., C.B.E., O.B.E. (mil.), Sc.D., F.R.I.C., F.Inst.P., F.R.S., The Old Vicarage, Antony, Torpoint, Cornwall
*1910 Atkinson, G. T., Gresham House, Esplanade, Lowestoft, Suffolk
1951 Atlantic Biological Station, St Andrews, N.B., Canada

1948 Baal, H. J., 3 Bel Royal Villas, Jersey, C.I.
1950 Baerends, Prof. G. P., Zoölogisch Laboratorium, Rijksstraatweg 78, Haren (Gron.), Holland
1949 Bagenal, T. B., Marine Station, Millport, Isle of Cumbrae, Scotland
1956 Bailey, Miss J. A., 110 Cambridge Road, North Harrow, Middx
*1952 Baily, Joshua L. Jr., 4435 Ampudia Street, San Diego 3, California, U.S.A.
1950 Bainbridge, R., Ph.D., 43 Strathmore Avenue, Hull
1953 Bainbridge, V., West African Fisheries Research Institute, Freetown, Sierra Leone
*1920 Baker, J. R., D.Sc., Dept. of Zoology and Comparative Anatomy, University Museum, Oxford
1936 Baldwin, Prof. E., Ph.D., Dept. of Biochemistry, University College, Gower Street, London, W.C. 1 (**Council**, 1946–48)
1950 Ballantine, Miss D., The Laboratory, Citadel Hill, Plymouth, Devon
1955 Ballantine, W. J., A3 Downing College, Cambridge
1953 Barker, J. A., 27 Finchley Way, Finchley, London, N. 3
1949 Barnard, E. E. P., 7 Webster Gardens, Ealing, London, W. 5
1956 Barnard, J. Laurens, Ph.D., Allan Hancock Foundation, University of California, Los Angeles 7, California, U.S.A.
1939 Barnes, H., D.Sc., F.R.I.C., Marine Station, Millport, Isle of Cumbrae, Scotland
1954 Barnes, M. McC., Mandeville, Rosebank Crescent, Pennsylvania, Exeter, Devon
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1956 Hardy, M. G., Dept. of Zoology, The University, Reading
1950 Harley, Miss M. B., Dept. of Zoology, Queen Mary College, Mile End Road, London, E. 1
1932 Harris, Prof. J. E., Ph.D., F.R.S., Dept. of Zoology, The University, Bristol (**Council**, 1946–49, 1950–53, 1954→)
1939 Harrison, Prof. R. J., D.Sc., M.R.C.S., L.R.C.P., Vinicombe, The Woodlands, Farnborough, Kent
1929 Hart, T. J., D.Sc., National Institute of Oceanography, Wormley, near Godalming, Surrey

1934 Hartley, The Rev. P. H. T., Badingham Rectory, Woodbridge, Suffolk
1955 Hartman, Dr W. D., Peabody Museum of Natural History, Yale University, New Haven, Conn., U.S.A.
1924 Harvey, H. W., Sc.D., F.R.S., The Laboratory, Citadel Hill, Plymouth, Devon
1933 Harvey, Prof. L. A., Dept. of Zoology, The University, Exeter, Devon. (**Council**, 1940–43)
1951 Harvey, Mrs M. S. J., 12 Trevarrick, St Austell, Cornwall
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1950 Haswell, G. A., 23 Russell Avenue, Hartley, Plymouth, Devon
1954 Hatley, Miss J., 1659 Pershore Road, Cotteridge, Birmingham 30
1952 Hawes, F. A. B., R.O.S.C.M. Ltd., Research Laboratory, 46 New Quay Road, Hamworthy, Poole, Dorset
1939 Hayes, Dr F. R., Dalhousie University, Halifax, N.S., Canada
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1950 Hazevoet, A. C., Vogelenzangstraat 19–I, Amsterdam West, Holland
1954 Hedgpeth, Dr Joel W., Scripps Institution of Oceanography, La Jolla, California, U.S.A.
1948 Hedley, Ronald H., Ph.D., British Museum (Natural History), Cromwell Road, London, S.W. 7
1955 Heezen, B. C., Lamont Geological Observatory, Palisades, New York, U.S.A.
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1953 Henderson, Mrs W. M., 37 Moor Lane, St Budeaux, Plymouth, Devon
1925 Hentschel, C. C., 7 Dudley Court, Upper Berkeley Street, London, W. 1
1952 Hepper, B. T., Elmfield, Combe-in-Teignhead, Newton Abbot, Devon
1950 Herring Industry Board, 1 Glenfinlas Street, Edinburgh 3
1939 Hewer, H. R., Assistant Professor, Dept. of Zoology, Imperial College of Science, London, S.W. 7
1955 Hewitt, Miss C. E., Fiershill Farm, Trysull, near Wolverhampton, Staffs
1926 Hickling, C. F., C.M.G., Sc.D., Colonial Office, Sanctuary Buildings, Great Smith Street, London, S.W. 1. (**Council**, 1947–50)
1951 Hidaka, Prof. K., Geophysical Institute, Tokyo University, Tokyo, Japan.
1926 Hill, Prof. A. V., C.H., O.B.E., Sc.D., LL.D., F.R.S., 16 Bishopswood Road, Highgate, London, N. 6. (**Council**, 1925–29, 1930–33, 1934–37, 1938–41, 1942→; representing Royal Society, 1944–55; **Vice-President**, 1948–55; **President**, 1955→)
*1949 Hill, D. K., Postgraduate Medical School of London, Ducane Road, London, W. 12
1947 Hill, M. N., Ph.D., 11 Chaucer Road, Cambridge. (**Council**, 1956→)
*1921 Hindle, E., Sc.D., F.R.S., 51 Warwick Avenue, London, W. 9. (**Council**, 1946–49; representing British Association, 1950→)
1937 Hinton, M. A. C., F.R.S., Glaister, Wrington, near Bristol, Somerset
1926 Hobson, Prof. A. D., King's College, Newcastle-upon-Tyne 1, Northumberland
1954 Hobson, G. E., 3A Western Court, Western Road, Bournemouth, Hants
1948 Hockley, A. R., Dept. of Zoology, The University, Southampton
1939 Hodgkin, Prof. A. L., F.R.S., Physiological Laboratory, Cambridge (**Council**, 1951–52, 1953–56)
*1953 Hoestlandt, Prof. H., D.Sc., 13 rue de Toul, Lille (Nord), France
1952 Holden, M. J., W.A.F.R.I., Field Station, Birnin Kebbi, Sokoto Province, Nigeria

1954 HOLLAND: The Director, Koninklijk Zoologisch Genootschap, Natura Artis Magistra, Amsterdam
1956 HOLLIDAY, F. G. T., 43 Maple Road, Rubery, near Birmingham
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1948 HOLSGROVE, H. E., 121 Eden Bridge Road, Bush Hill Park, Enfield, Middx
1950 HONEKER, C., 59 Uplands, Alkrington, Middleton, Manchester
*1954 HOPE, D. B., Dept. of Pharmacology, South Parks Road, Oxford
1956 HOPSON, A., Marine Biological Station, Port Erin, Isle of Man
1951 HORNBY, Miss C. E., 53 Langley Drive, Wanstead, London, E. 11
1933 HORNE, F. R., National Institute of Agricultural Botany, Huntingdon Road, Cambridge
1950 HORRIDGE, G. A., Ph.D., Gatty Marine Laboratory, The University, St Andrews, Fife
1955 HOUGHTON, D. R., 7 Muriel Road, Waterlooville, Hants
1950 HOWARD, G. H., 14 Westland Avenue, Bargate, Grimsby, Lincs
1948 HOWE, Surg.-Cdr. D.C., R.N., c/o 80 Lumsden Avenue, Shirley, Southampton
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1953 HOWELLS, Dr W. R., 60 James Street, Llanelly, Carms
1932 HOWES, N. H., Dept. of Zoology, University College, Gower Street, London, W.C.1
1952 HOYLE, G., Dept. of Zoology, The University, Glasgow, W. 2
1955 HUDSPITH, W. H., 1 King's Avenue, Rochester, Kent
*1954 HUGHES, A., Sc.D., Anatomy School, Cambridge
1953 HULL: THE LIBRARIAN, University College, Hull
1928 HUNT, O. D., Corrofell, Newton Ferrers, Devon. (**Council**, 1944–47, 1948–51, 1952–55, 1956→)
1947 HUNTER, W. RUSSELL, Dept. of Zoology, The University, Glasgow, W. 2
*1947 HURRELL, H. G., J.P., Moorgate, Wrangaton, Devon
*1952 HUTTON, ROBERT F., Ph.D., State Board of Conservation Marine Laboratory, Maritime Base, Bayboro Harbor, St Petersburg, Florida, U.S.A.

1946 ICELAND: ATVINNUDEILD HÁSKÓLANS (FISKIDEILD), Reykjavik, Iceland
1956 ILLG, Associate Prof. P. L., Dept. of Zoology, University of Washington, Seattle 5, Wash., U.S.A.
1945 IMPERIAL CHEMICAL INDUSTRIES, LTD., Nobel House, 2 Buckingham Gate, London, S.W. 1
1954 INDONESIA: THE PROFESSOR OF ZOOLOGY, University of Indonesia, Djalan Taman Sari 64, Bandung, Java
1952 INGLIS, W. G., British Museum (Natural History), Cromwell Road, London, S.W. 7
1953 INSTITUTE OF SEAWEED RESEARCH, Inveresk, Midlothian

1952 JACKMAN, L. A. J., 44 Old Torquay Road, Preston, Paignton, Devon
1954 JAGER, J. C., van Reesstraat 122, The Hague, Holland
1953 JAGGER, J. R., 5 Duckworth Street, Stoke, Devonport, Devon
1952 JEAN, Prof. YVES, Fisheries Research Station, P.O. Box No. 531, Colombo 3, Ceylon
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1952 Jeffery, F. J., Plymouth College, Plymouth, Devon
1953 Jellard, Dr C. H., B.M., Public Health Laboratory, South Devon and East Cornwall Hospital, Greenbank Road, Plymouth, Devon
1935 Jenkin, Miss P. M., Dept. of Zoology, The University, Bristol
1934 Jepps, Miss M. W., D.Sc., 17 Manor Court, Grange Road, Cambridge
1937 Jersey: Conservateur honoraire du Musée de la Société Jersiaise
*1924 Jesus College, Oxford
*1947 John, C. C., D.Sc., Dept of Marine Biology and Fisheries, Beach P.O., Trivandrum, S. India
*1947 Johnson, D. S., 10 St John's Road, Cambridge
1944 Johnson, Dr F. R., Osu Fisheries Station, P.O. Box 630, Accra, Gold Coast Colony
1956 Jones, B. W., 62 Great Bushey Drive, Totteridge, London, N. 20
*1948 Jones, C. Burdon, Ph.D., Dept. of Zoology, University College of N. Wales, Bangor, Caern
*1950 Jones, E. Idris, M.D., 6 Kewferry Road, Northwood, Middx
*1949 Jones, Prof. E. W. Knight, Ph.D., Dept. of Zoology, University College, Swansea
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1948 Jones, J. D., Nantyci, Llysonnen Road, Carmarthen, S. Wales
1946 Jones, L. W. G., Cumber House, Manor Road, Brixham, Devon
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1953 Jones, P. G. W., Woodberry, Tolmers Road, Cuffley, Herts
1949 Jones, R. J., 17 Haling Park Road, South Croydon, Surrey
1936 Jones, Rodney R. M., Tros-yr-Afon, Penmon, Anglesey
1946 Jones, Prof. R. V., C.B., C.B.E., D.Phil., F.Inst.P., Dept. of Natural Philosophy, Marischal College, Aberdeen
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1950 Juniper, A. J., The Polytechnic, Regent Street, London, W. 1

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1954 Kain, Miss J. M., 11 Acol Road, London, N.W. 6
1951 Kampa, E. M., Ph.D. (Mrs B. P. Boden), Scripps Institution of Oceanography, La Jolla, California, U.S.A.
1954 Karnatak University: Karnatak University Library, Dharwar (State Bombay), India
1956 Kasturirangan, L. R., Central Fisheries, Ernakulam P.O., S. India
1945 Katz, Max, Ph.D., c/o Prof. R. E. Dimick, Dept. of Fish and Game Management, Oregon State College, Corvallis, Oregon, U.S.A.
1951 Kay, Miss E. A., Dept. of Zoology, University of Hawaii, Honolulu, Hawaii, U.S.A.
1955 Keady, P. J., 31 Seapoint Avenue, Blackrock, Dublin, Ireland
1940 Keilin, Prof. D., Sc.D., F.R.S., Molteno Institute, Cambridge. (**Council**, 1940–43)

1950 Keir, Ronald S., Newfoundland Fisheries Research Station, Water Street East, St Johns, Newfoundland
1955 Kellaway, Mrs R., 18 Thornyville Villas, Oreston, near Plymouth, Devon
1946 Kelley, D. F., Pear Tree Cottage, Cheapside, Horsell, Woking, Surrey
1954 Kelly, A. L., 352 Nautilus, La Jolla, California, U.S.A.
1949 Kennedy, G. Y., Ph.D., F.R.I.C., Dept. of Cancer Research, University Field Laboratory, Blackbrook Road, Sheffield 10
1946 Kenya: The Game Warden, Game Department, P.O. Box 241, Nairobi
*1952 Kerkut, G., Sub-Dept. of Physiology, The University, Southampton
*1954 Kesteven, Dr G. L., c/o F.A.O.U.N. Viale delle Terme di Caracalla, Rome, Italy
1950 Keynes, R. D., Ph.D., Physiological Laboratory, Cambridge
1949 Kimmins, B. J., P.O. Box 603, Addis Ababa, Ethiopia
1928 King, Mrs A. Redman, Mixton House, Lerryn, Lostwithiel, Cornwall
1947 Kingsbridge Modern Secondary School, Kingsbridge, Devon
1952 Kings' College: Dept. of Zoology, Newcastle upon Tyne 1
1954 Kingwell, M. C., Beechfield, South Brent, S. Devon
1927 Kirtisinghe, P., Dept. of Zoology, University of Ceylon, Colombo 3, Ceylon
1950 Kisch, B. S., C.I.E., 38 Boulevard Thiers, St Jean-de-Luz, B.P., France
1956 Kitamura, Hiroyuki, Kobe Marine Observatory, Kobe, Japan
1930 Kitching, J. A., O.B.E., Ph.D., Dept. of Zoology, The University, Bristol
1939 Knight, Miss Margery, D.Sc., University Hall for Woman Students, Holly Road, Fairfield, Liverpool. (**Council**, 1943–46)
1955 Knott, C. C., 76 Pasley Street, Stoke, Plymouth, Devon
1945 Knowles, Sir Francis G. W., Bt., D.Phil., Marlborough College, Marlborough, Wilts
1955 Knox, G. A., Zoology Dept., Canterbury University College, Christchurch, New Zealand
1955 Kohn, A. J., Hawaii Marine Laboratory, University of Hawaii, Honolulu 14, Hawaii, U.S.A.
1950 Korn, M. E., 62 Aberdare Gardens, Hampstead, London, N.W. 6
1949 Kristensen, Dr Ingvar, Zoölogisch Station, den Helder, Holland
*1954 Krogh, Erik, M.D., Associate Prof., Normal Anatomist Institut, Aarhus Universitet, Aarhus, Denmark
1955 Kruse, P. J., Jr., Marine Laboratory, University of Miami, Coral Gables 34, Florida, U.S.A.

1954 Lacey, A. J., 24 The Gardens, Monkseaton, Northumberland
1955 Lade, Miss B. I., Southdown House, Ewhurst, near Guildford, Surrey
1956 Lance, Miss J., Dept. of Zoology, The University, Southampton
1953 Landa, Antonio, P.O. Box 3665, Balboa, Canal Zone, S. America
1950 Lasker, Reuben, Scripps Institution of Oceanography, La Jolla, California, U.S.A.
1956 Laverack, M. S., Sub-Dept. of Physiology, The University, Southampton
*1925 Lebour, Miss M. V., D.Sc., Kean Hill, Cawsand, near Plymouth, Devon
1947 Leehane, J. D. B., 14 Wyndham Street East, Plymouth, Devon
1955 Leivestad, H., Klostergt 2, Bergen, Norway
1935 LeMare, D. W., Fisheries Dept., Federation of Malaya and Singapore, Penang, Malaya
*1954 Leston, D., 44 Abbey Road, London, N.W. 8

1956 LeSueur, R. F., The Museum, Jersey, C.I.
1951 Lewis, J. B., The Bellairs Research Institute of McGill University, St James, Barbados, B.W.I.
1956 Lewis, J. G. E., Hawkesden, Yew Lane, East Grinstead, Sussex
1952 Lewis, J. R., Dept. of Zoology, The University, Leeds
1951 Lightowlers, F., 60 Harberton Park, Belfast, N. Ireland
1954 Lilly, S. J., Ph.D. (Mrs Gilpin-Brown), Church Knowle, near Wareham, Dorset
1956 Ling, J. K., C.S.I.R.O., Division of Fisheries and Oceanography, Cronulla, N.S.W., Australia
1932 Lisbon: Instituto de Biologia Maritima, Cais do Sodré, Lisbon, Portugal
1955 Llewellyn, J., Ph.D., Dept. of Zoology and Comparative Physiology, The University, Edgbaston, Birmingham 15
1950 Longhurst, A. R., Landford Cottage, Landford, Wilts
1953 Longton, P. A., 37 Headland Park, North Hill, Plymouth, Devon
1954 Lorraine, N. S. R., M.D., Ch.B., D.P.H., F.R.S.E., Public Health Department, London Road, Hadleigh, Essex
1948 Lovegrove, T., Marine Laboratory, Victoria Road, Torry, Aberdeen
1953 Lowe, Mrs E. K., 27a Greenbank Road, Ambleside, Westmorland
1950 Lowenstein, Prof. O. E., D.Sc., Ph.D., F.R.S., Dept. of Zoology, The University, Birmingham 15. (**Council**, 1952–55)
1950 Lowy, J., Ph.D., Dept. of Zoology, Queen's University, Belfast, N. Ireland
1931 Lucas, C. E., C.M.G., D.Sc., Marine Laboratory, Victoria Road, Torry, Aberdeen. (**Council**, 1949–52, 1954→)
1956 Lunz: Biologische Station Lunz, Lunz am See, Niederösterreich, Austria
1953 Lynch, Dr G. A. C., Beeches, Crapstone, Yelverton, Devon
1938 Lysaght, Miss A. M., Ph.D., 6 Cumberland Gardens, London, W.C. 1

1938 MacDonald, R., 112 Antrim Road, Belfast, N. Ireland
1935 Mackenzie, Col. W., O.B.E., c/o Messrs Peel & Co., Ltd., P.O. Box 331, Alexandria, Egypt
1929 Mackinnon, Prof. D. L., D.Sc., 44 St Leonard's Terrace, Chelsea, London, S.W. 3. (**Council**, 1938–42)
1937 Mackintosh, N. A., C.B.E., D.Sc., 7 Hinde House, Hinde Street, London, W. 1. (**Council**, 1946–49, 1951–54, 1955→)
1947 Macnae, William, Dept. of Zoology, Rhodes University College, Grahamstown, S. Africa
*1954 Maddocks, Miss A. C., 28 Denison Road, Victoria Park, Manchester 14
1956 Madeira: Museu Municipal do Funchal, Madeira
*1925 Magdalen College, Oxford
1955 Mahakoshal Mahavidyalaya (The Principal), Jabalpur, India
1951 Malkin, J. J. S., 33 Elton Parade, Darlington
1948 Mansfield, A. W., Dept. of Zoology, McGill University, Montreal, Quebec, Canada
1952 Mansfield, Lt.-Col. K. F., Luckwell Bridge, Wheddon Cross, Minehead, Som
1955 Mansueti, R. J., Maryland Dept. of Research and Education, Chesapeake Biological Laboratory, Solomons, Maryland, U.S.A.
*1928 Manton, S. M., Sc.D., F.R.S. (Mrs J. P. Harding), 18 Ennerdale Road, Richmond, Surrey
1953 Manwaring, Miss R. A., Dept. of Zoology, The University, Exeter, Devon

1951 MARINE BIOLOGY STATION, University College of North Wales, Menai Bridge, Anglesey
1956 MARKOWSKI, S., D.Sc., Central Electricity Authority, The Biological Research Laboratory, Grove Road Generating Station, Lodge Road, London, N.W. 8
1951 MARSHALL, C. J., M.D., F.R.C.S., Penhaven, The Drive, Mevagissey, Cornwall
1949 MARSHALL, DOUGLAS, M.P., Lanwithan House, Lostwithiel, Cornwall
1951 MARSHALL, K. E., The Ferry House, Far Sawrey, Ambleside, Westmorland
*1947 MARSHALL, Miss S. M., D.Sc., Marine Station, Millport, Isle of Cumbrae, Scotland
1953 MARSHALL, P. T., The Priory, Kingswear, Devon
1955 MARTIN, A. L., 22 Luttrell Avenue, Putney, London, S.W. 15
1951 MASON, J., Marine Laboratory, Victoria Road, Torry, Aberdeen
1939 MATTHEWS, L. HARRISON, Sc.D., F.R.S., Zoological Society of London, Regent's Park, London, N.W. 1. (**Council**, 1944–47, 1949–52)
*1947 MATTHEWS, Mrs J. B., Ph.D., (Miss G. C. Evans), The Rock Garden, Gerrans, Portscatho, Cornwall
1954 MAUCHLINE, J., 4 The University, Glasgow, W. 2
1937 MAYNE, CYRIL F., O.B.E., F.R.C.S., c/o Barclays Bank Ltd., Plymouth, Devon
1953 MAYNE, R. B., Martins Meadow, Yelverton, Devon
1949 MCCARTHY, Miss D. F., 70 Halton Terrace, Kensington Park, Adelaide, S. Australia
1951 MCCARTON, Miss L., 89 Eglantine Avenue, Malone Road, Belfast, N. Ireland
1910 MCCLEAN, Capt. W. N., 39 Phillimore Gardens, London, W. 8
*1929 MCEWEN, Mrs LAWRENCE, Isle of Muck, Isle of Eigg, Inverness-shire
*1952 MCGILL, D. A., 252 Terrace Park, Rochester 11, New York, U.S.A.
*1954 MCGOWAN, J. A., Trust Territory of the Pacific Islands, Office of the Staff Marine Biologist, Koror, Caroline Islands
1955 MCINNES, Miss F. R. B., 65 Gwendoline Avenue, Upton Park, London, E. 13
1948 MCINTYRE, A. D., Marine Laboratory, Victoria Road, Torry, Aberdeen
1950 MCINTYRE, J. M., Greenfield, Cramer Street, Stafford, Staffs
1953 MENKER, DONALD F., Marine Laboratory, University of Miami, Coral Gables, Florida, U.S.A.
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1954 MENZIES, Dr R. J., Lamont Geological Observatory, Torrey Cliff, Palisades, New York, U.S.A.
1953 MERCER, B. V., Blakewell House, Painswick, Glos
1953 MEREDITH, Miss H. M., 7 The Lynchets, Lewes, Sussex
1954 MERRETT, M. G., 22 Fullerton Road, Milehouse, Plymouth, Devon
1950 MESSENGER, K. G., Uppingham School, Rutland
1951 MESSENT, R. K., 13 Sedberg Street, Preston, Lancs
1939 METROPOLITAN WATER BOARD, 177 Rosebery Avenue, London, E.C. 1
1950 MEXICO: DEPARTMENT OF PHYSIOLOGY, NATIONAL UNIVERSITY, Apartado Postal 13699, Mexico, D.F.
1947 MIAMI UNIVERSITY MARINE LABORATORY, Coral Gables 34, Florida, U.S.A.
1951 MIJ TOT EXPLOITATIE VAN KOOKSOVENGASSEN (Mekog), Ijmuiden, Holland
1923 MILFORD HAVEN TRAWLER OWNERS ASSOCIATION LTD., Milford Haven, Pembs
1949 MILLAR, R. H., Ph.D., Marine Station, Millport, Isle of Cumbrae, Scotland
1946 MILLER, CYRIL J., 16 Beechwood Terrace, Mutley, Plymouth, Devon

1956 MILLER, M. C., Marine Biological Station, Port Erin, Isle of Man
1955 MILLER, P. L., Downing College, Cambridge
1955 MILLIGAN, W., Sgts. Mess, A.P.L., Singapore Lines, Khormaksar, Aden
1949 MILLOTT, Prof. N., Ph.D., Dept. of Zoology, Bedford College, Regent's Park, London, N.W. 1
1953 MILLS, D. H., Atlantic Biological Station, St Andrews, New Brunswick, Canada
1949 MISTAKIDIS, M. N., Fisheries Laboratory, Burnham-on-Crouch, Essex
1947 MITRA, G. N., Assistant Director of Fisheries, Cuttack (Orissa), India
1952 MOHAMED, Prof. ABDEL FATTAH, Ph.D., Oceanography Dept., Faculty of Science, Alexandria University, Maharram Bey, Alexandria, Egypt
1951 MOHR, J. L., Ph.D., Dept. of Zoology, University of Southern California, Los Angeles 7, Calif., U.S.A.
1956 MOLLOY, Miss F. M., Dept. of Botany and Zoology, Chelsea Polytechnic, London, S.W. 3
1940 MOORE, HILARY B., Ph.D., Marine Laboratory, Miami University, Coral Gables, Florida, U.S.A.
1955 MORGAN, Miss R. B., 37 Fidlas Avenue, Llanishen Cardiff
1949 MORTON, J. E., Ph.D., Dept. of Zoology, Queen Mary College, Mile End Road, London, E. 1
1953 MOSS, Miss M. P., Lady Margaret Hall, Oxford
1931 MOUNT DESERT ISLAND BIOLOGICAL LABORATORY, Salisbury Cove, Maine, U.S.A.
1942 MOYNAHAN, Dr E. J., Wayside, Jordans, near Beaconsfield, Bucks.
1953 MULLER, R. L., 38 Brendon Street, London, W. 1
1952 MUNN, R. B., 11002 East Saragossa Street, Whittier, Calif., U.S.A.
1955 MURRAY, R. W., Ph.D., Dept. of Zoology and Comparative Physiology, The University, Edgbaston, Birmingham, 15

1955 NAGABHUSHANAM, R., Dept. of Zoology, Andhra University, Visakhapatnam, India
1952 NAYLOR, E., Officers' Mess, R.A.F., Kirkham, Lancs.
1955 NAYLOR, Miss MARGARET, Ph.D., Dept. of Botany, The University, Hull
1933 NEALE, MORLEY H., C.B.E., Chaffcombe House, Chard, Somerset. (**Council**, 1934–36, 1939–42, 1943–46, 1947–50; **Vice-President**, 1951→)
1939 NEEDHAM, JOSEPH, Sc.D., F.R.S., Gonville and Caius College, Cambridge
1953 NEWELL, B. S., I.N.F.R.O., P.O. Box 754, Zanzibar
1947 NEWELL, G. E., Ph.D., Dept. of Zoology, Queen Mary College, Mile End Road, London, E. 1. (**Council**, 1955→)
1951 NEWFOUNDLAND FISHERIES RESEARCH STATION, Water Street East, St John's, Newfoundland
1947 NEWTON, Prof. LILY, D.Sc., Dept. of Botany, University College of Wales, Aberystwyth, Cardigan. (**Council**, 1947–50, 1955→)
1930 NICHOLLS, A. G., Ph.D., C.S.I.R.O., Division of Fisheries, Stowell, Hobart, Tasmania
1950 NICOL, J. A. C., D.Phil., Woodview, Tideford, near Saltash, Cornwall
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1953 NORDLI, O., Statens Fiskarfagskule, Florø, Norway
1949 NORRIS, E., Dept. of Biology, Ewell Technical College, Reigate Road, Ewell, Surrey

1951 North Carolina, University of, Institute of Fisheries Research, Box 629, Morehead City, N. Carolina, U.S.A.
1950 North, J., 316 Lee High Road, Lewisham, London, S.E. 13
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1948 Nunn, S. M., The Laboratory, Citadel Hill, Plymouth, Devon
1950 Nurse, F. R. (Mrs R. M. Allison), Dept. of Biology, Canterbury University College, Christchurch, New Zealand

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1947 Oldfield, Mrs E., County Technical College, Guildford, Surrey
1938 Oliver, James H., Ph.D., 16 Southwark Street, London, S.E. 1
1937 Omer-Cooper, J., Dept. of Zoology, Rhodes University College, Grahamstown, S. Africa
1939 Ommanney, F. D., Ph.D., Fisheries Research Station, P.O. Box 749, Singapore
1949 Orkin, P. A., Natural History Dept., Marischal College, Aberdeen
1947 Orr, A. P., D.Sc., Marine Station, Millport, Isle of Cumbrae, Scotland
1945 Otago University Library, Dunedin, N.I., New Zealand
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1950 Owen, G., Dept. of Zoology, The University, Glasgow, W. 2
1953 Owen, Miss G., Rustington, Flat 2, St Wilfred's Road, Worthing
1949 Owre, Mrs Harding B., Marine Laboratory, University of Miami, Coral Gables 34, Florida, U.S.A.
1951 Oxford: Department of Zoology and Comparative Anatomy, University Museum, Oxford

1951 Pacific Biological Station, Nanaimo, B.C., Canada
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1953 Pampapathi-Rao, K., Ph.D., Dept of Zoology, S.V. University, Tirupathi, S. India
*1939 Panikkar, N. K., D.Sc., Central Marine Fisheries Station, Mandapam Camp P.O., S. India
*1923 Pantin, C. F. A., Sc.D., F.R.S., Trinity College, Cambridge. (**Council**, 1930–32, 1933–36, 1937–40, 1941–44, 1945–49, 1951–53; representing Cambridge University, 1946–49, 1951–53)
1951 Papworth, A. H., 6 Ashford Avenue, Ashford, Middx
1941 Parke, Miss M. W., D.Sc., The Laboratory, Citadel Hill, Plymouth, Devon
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1954 Parrish, B. B., Marine Laboratory, Victoria Road, Torry, Aberdeen
1937 Parry, D. A., Ph.D., Dept. of Zoology, Downing Street, Cambridge
1950 Parry, Miss G., Dept. of Zoology, Bedford College, Regent's Park, London, N.W. 1
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1910 Plymouth Education Authority, Cobourg Street, Plymouth, Devon
1906 Plymouth, Port of, Incorporated Chamber of Commerce, Plymouth, Devon
1924 Plymouth Proprietary Library, 1 Alton Terrace, North Hill, Plymouth, Devon
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*1932 Rothschild, The Hon. Miriam (The Hon. Mrs George Lane), The Cottage, Ashton Wold, Peterborough, Northants. (**Council**, 1942–45)
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